REMARKS

I. The Substitute Specification and the October 2003 and June 2004 Preliminary Amendments

The Office Action indicates that the substitute specification submitted June 20, 2001 has not been entered because it does not conform to 37 C.F.R. 1.125(b) and (c) (1) because it does not contain a statement as to a lack of new matter and (2) because a marked up copy of the specification has not been supplied. In response, Applicants submit herein a substitute specification and a marked up copy showing the changes made. In addition, in accordance with 37 C.F.R. § 121, Applicants (1) add a paragraph to refer to the priority applications; and (2) add the text of a Sequence Listing into the specification. Pursuant to 37 C.F.R. § 1.121(f) and 37 C.F.R. § 1.825, Applicants assert (1) that the subject matter of the amendments are supported by the application as filed and (2) that the amendments do not contain new matter.

The Office Action also indicates that the Preliminary Amendment submitted October 3, 2003 and the Preliminary Amendment submitted June 30, 2004 have not been entered. Applicants request that the Examiner enter the Amendments submitted October 3, 2003 and June 30, 2004.

The Office Action also indicates that replacement sheet Fig. 1 was not included with the October 3, 2003 filing. Applicants' representative has reviewed its file and has determined that the replacement sheet for Fig. 1 was included in its copy of the filing. In light of the submission herein, however, Applicants believe that this matter is moot.

The Office Action further notes the following discrepancies with regard to the substitute drawings submitted June 20, 2001:

1. Substitute Fig. 4 omits panel B of original Fig. 5;
2. Original Fig. 1 was omitted;
3. SEQ ID NO:2 does not match the description of SEQ ID NO:2 in the original specification as being nucleotides 11-1447 of the nucleotide sequence of original Fig. 1 (thus requiring a submission of substitute computer readable and paper copy of Sequence Listing).

In response to the enumerated points above, Applicants:

1. Resubmit the figures as filed in the PCT to include panel B of original Fig. 5;

2. Resubmit the figures as filed in the PCT to include original Fig. 1;
3. Resubmit copies of the computer readable and paper Sequence Listings filed on June 28, 2004. Applicants believe that these Sequence Listings are correct and in compliance with PTO practice.

II. The February 26, 2002 Information Disclosure Statement

The Office Action indicates that references 12, 22 and 49 cited in the Information Disclosure Statement submitted February 26, 2002 have not been considered because a copy of each was not submitted. In response, Applicants submit copies of references 12, 22 and 49 herein.

III. Figure 2

The Office Action requires submission of new corrected drawings, in particular due to the quality of Fig. 2 submitted with the international application. In response, Applicants submit a Substitute Fig. 2 herein.

IV. The Claim Objections

The Office Action makes a number of objections to claims 2-4, 9-12, 16-18 and 40. In response, the claims are amended to obviate any basis for the objections. Reconsideration and withdrawal of the objections are respectfully requested.

V. The Rejections Under 35 U.S.C. § 101/§ 112

The Office Action rejects claims 1-4 under 35 U.S.C. § 101, asserting that the claims read on products of nature. In response, claim 1 is amended to refer to an "isolated" nucleic acid sequence as suggested.

The Office Action rejects claims 12 and 16 under 35 U.S.C. § 101 and 35 U.S.C. § 112, asserting that "the specification does not contain an assertion of what the medicament or pharmaceutical composition would be used to treat." In order to expedite prosecution of this application, Applicants have canceled claims 12 and 16. Applicants reserve the right to file divisional application(s) directed to the subject matter of claims 12 and 16.

Reconsideration and withdrawal of the rejection under 35 U.S.C. § 101/§ 112 are respectfully requested.

VI. The Rejection Under 35 U.S.C. § 112

The Office Action rejects claim 40 under 35 U.S.C. § 112, first and second paragraph. In order to expedite prosecution of this application, Applicants have canceled claim 40. Applicants reserve the right to file divisional application(s) directed to the subject matter of claim 40.

The Office Action rejects claim 1, 2, 9-12, 16-18 and 40 under 35 U.S.C. § 112, second paragraph, for use of the term "functional equivalent, derivative or bioprecursor thereof." Reconsideration and withdrawal of the rejection under 35 U.S.C. § 112 are respectfully requested. In order to expedite prosecution of this application, Applicants have amended claim 1 as suggested. Applicants reserve the right to file divisional application(s) directed to the subject matter of claim 1 as originally filed.

Reconsideration and withdrawal of the rejection under 35 U.S.C. § 112 are respectfully requested.

VII. The Indication of Allowable Subject Matter

Applicants greatly appreciate the indication that claims 1-4, 9-11, 17 and 18 would be allowable if rewritten to overcome the objections and rejections under 35 U.S.C. §§ 101 and 112.

VIII. Conclusion

Early consideration and prompt allowance of the pending claims are respectfully requested. Should the Examiner require anything further, the Examiner is invited to contact Applicants' representative at the telephone number below.

Respectfully submitted,

By Laura A. Donnelly

Laura A. Donnelly
Reg. No. 38,435

Johnson & Johnson
One Johnson & Johnson Plaza
New Brunswick, NJ 08933-7003
(732) 524-1729
Dated: August 29, 2005

Enclosures:
Substitute Specification
Marked Up Copy of Substitute Specification
Figs. 1-8 (includes Figs. as originally filed in PCT application)
June 28, 2004 Submission
Substitute Fig. 2
References 12, 22 and 49 cited in February 26, 2002 Information Disclosure Statement

Serial No. 09/869,079 Docket No. JAB-1458 By: UAU

Application of: Maguire et al Mailed: August 29, 2005

Entitled: Human IAKt-7

THE FOLLOWING HAS BEEN RECEIVED IN THE U.S. PATENT OFFICE ON THE DATE STAMPED HEREON:

- ☐ Oath or Declaration
- ☐ Assignment • Refs. 12, 22 + 49 cited in 2/26/02 IDS
- ☐ Response
- ☐ Fee Transmittal
- ☒ Charge to Deposit Account 10-0750
- ☒ Amendment
- ☐ Extension of Time
- ☐ Issue Fee Transmittal
- ☐ PCT Filing
- ☐ IDS-Form 1449
- • Copy of June 28, 2004 submission
- ☒ ~~Drawings~~ Figs. 1-8 ~~sheets~~ , substitute Fig. 2
- ☐ MPEP 609/
- ☐ Notice of Appeal
- ☐ Brief
- ☐ Priority Document
- ☐ Status Inquiry
- ☐ Sequence Listings/Diskette
- ☐ Biological Deposit Declaration
- ☒ Other Substitute Specification + marked-up copy of substitute specification

COPY

Serial No. 09/869,079 Docket No. JAB-1458 By: LAO

Application of: Masure et al. Mailed: June 28, 2004

Entitled: HUMAN AKT-3

THE FOLLOWING HAS BEEN RECEIVED IN THE U.S. PATENT OFFICE ON THE DATE STAMPED HEREON:

- ☐ Oath or Declaration
- ☐ Assignment
- ☒ Response (Request for Reconsideration on Petition) and supporting documentation including cover letter
- ☐ Fee Transmittal
- ☐ Charge to Deposit Account 10-0750
- ☒ Supplemental Preliminary Amendment
- ☐ Extension of Time
- ☐ Issue Fee Transmittal
- ☐ PCT Filing
- ☐ IDS-Form 1449
- ☐ Drawings ____ sheets
- ☐ MPEP 609/
- ☐ Notice of Appeal
- ☐ Brief
- ☐ Priority Document
- ☐ Status Inquiry
- ☒ Sequence Listings/Diskette + verification statement
- ☐ Biological Deposit Declaration
- ☐ Other

DT02 Rec'd PCT/PTO 30 JUN 2004

OIPE IAP88
AUG 3 1 2005
PATENT & TRADEMARK OFFICE

Serial No. 09/869,079 Docket No. JAB-1458 By: LAO

Application of: Masure et al. Mailed: June 28, 2004

Entitled: HUMAN AKT-3

THE FOLLOWING HAS BEEN RECEIVED IN THE U.S. PATENT OFFICE ON THE DATE STAMPED HEREON:

- ☐ Oath or Declaration
- ☐ Assignment
- ☒ Response (Request for Reconsideration on Petition) and supporting documentation, including cover letter
- ☐ Fee Transmittal
- ☐ Charge to Deposit Account 10-0750
- ☒ Supplemental Preliminary Amendment
- ☐ Extension of Time
- ☐ Issue Fee Transmittal
- ☐ PCT Filing ______
- ☐ IDS-Form 1449
- ☐ Drawings ____ sheets
- ☐ MPEP 609/______
- ☐ Notice of Appeal
- ☐ Brief
- ☐ Priority Document
- ☐ Status Inquiry
- ☒ Sequence Listings/Diskette + verification statement
- ☐ Biological Deposit Declaration
- ☐ Other ______

DT02 Rec'd PCT/PTO 30 JUN 2004

Serial No. 09/869,079 Docket No. JAB-1458 By: LAO

Application of: Masure et al. Mailed: June 28, 2004

Entitled: HUMAN AKT-3

THE FOLLOWING HAS BEEN RECEIVED IN THE U.S. PATENT OFFICE ON THE DATE STAMPED HEREON:

- ☐ Oath or Declaration
- ☐ Assignment
- ☒ Response (Request for reconsideration on petition) and supporting documentation including cover letter
- ☐ Fee Transmittal
- ☐ Charge to Deposit Account 10-0750
- ☒ Amendment supplemental preliminary
- ☐ Extension of Time
- ☐ Issue Fee Transmittal
- ☐ PCT Filing ____
- ☐ IDS-Form 1449
- ☐ Drawings ____ sheets
- ☐ MPEP 609/____
- ☐ Notice of Appeal
- ☐ Brief
- ☐ Priority Document
- ☐ Status Inquiry
- ☒ Sequence Listings/Diskette + verification statement
- ☐ Biological Deposit Declaration
- ☐ Other ____

Johnson & Johnson

OFFICE OF
SENIOR PATENT COUNSEL

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ldonnel2@corus.jnj.com

June 28, 2004

Mail Stop PCT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
Attention: PCT Legal Administration

Re: Renewed Petition Under 37 C.F.R. 1.137(b)
Our Docket No.: JAB-1458
Application No.: 09/869,079
Title: Human AKT-3
Applicants: Masure et al.

Dear Sir or Madam,

In response to the Decision of Petition mailed April 27, 2004 (copy enclosed), enclosed please find the following:

1. Request for Reconsideration of Decision on Petition Under 37 C.F.R. 1.137(b) and supporting documentation;
2. Supplemental Preliminary Amendment;
3. Substitute Sequence Listing together with Statement to Support Filing and Submission in accordance with 37 C.F.R. §§ 1.821-1.825.

Respectfully submitted,

Laura A. Donnelly

Laura A. Donnelly

LAD/kk
Encls.

Docket No. JAB-1458

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	MASURE et al.
Serial No.	:	09/869,079
Filed	:	I.A. 12/17/99
Title	:	HUMAN AKT-3
Art Unit	:	Unassigned
Examiner	:	Unassigned

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on

June 28, 2004
(Date of Deposit)

Laura A. Donnelly
(Name of applicant, assignee, or Registered Representative)

Laura A. Donnelly
(Signature)

June 28, 2004
(Date of Signature)

Mail Stop PCT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
Attention: PCT Legal Administration

REQUEST FOR RECONSIDERATION OF DECISION ON PETITION UNDER 37 C.F.R. 1.137(b)

Dear Sir:

Applicants hereby request reconsideration of the Decision on Petition Under 37 C.F.R. § 1.137(b) mailed April 27, 2004 ("Decision"). The following summarizes the series of events as set forth in the Decision and as supplemented by Applicants' representative herein (in bold):

1. 12/05/01 Notification of Missing Requirements mailed, required correction of Sequence Listing, paper copy and amendment in writing directing entry into specification.
2. 04/15/02 Response filed with a three-month extension of time, included Sequence Listing, paper copy and amendment in writing directing entry into specification.
3. 06/11/02 Notice of Acceptance of Application mailed.
4. 12/05/02 Communication that Notice of Acceptance was in error and therefore withdrawn and Notification of Defective Response ("Notification") mailed, required correction of Sequence Listing, paper copy and amendment in writing directing entry into specification. Non-extendable one-month period for reply set.
5. 01/05/03 due date for reply.
6. 10/13/03 Petition for Revival; Response to Notification of Defective Response; Preliminary Amendment; Sequence Listing, paper copy and Verification Statement received by U.S. Patent Office.
7. 12/04/03 Notice of Abandonment mailed. Stated that applicant failed to respond to 12/05/02 Notification of Defective Response.
8. **01/05/04 Applicants' representative contacted Winston M. Alvarado by telephone to check status of petition. Mr. Alvarado indicated that the Petition must not have matched with the file and asked that Applicants' representative fax a copy.**

9. **01/05/04 applicant faxed copy of 10/03/03 filing at Winston M. Alvarado's request. Communication indicated that copy of 10/3/03 date-stamped post card enclosed.**

The Decision states that the January 5, 2004 facsimile is the first date of filing of the October 3, 2003 submission. Applicants resubmit a complete copy of the October 3, 2003 filing herein, which demonstrates that the filing was actually received by the Patent Office on October 3, 2003.

The Decision states that although Applicants met the first and fourth requirements of 37 C.F.R. § 1.137(b), applicants did not provide a proper reply to the December 5, 2002 Notification. In particular, the Decision states (1) that the reply does not include a computer-readable copy of the latest substitute Sequence Listing; and (2) that the reply does not address all of the problems identified in the December 5, 2002 Notification. The Decision then states that the Comment Sheet attached to the Notification indicated (a) that sequences in lines 6-8 *and* lines 12-14 of page 25 of the specification were not included in the Sequence Listing and that the January 1, 2004 Amendment only provided SEQ ID NOS. for the sequences in lines 6-8 of page 25; and (b) that the amino acid sequence in line 36 of page 20 must also be included.

In response to all of the concerns set forth in the Decision, enclosed herewith are the following:

(1) a complete copy of the October 3, 2003 filing, which in turn includes:

(a) a copy of the date-stamped post card that demonstrates that the U.S. Patent Office received the filing on October 3, 2003;

(b) a copy of the Sequence Listing diskette received on October 3, 2003;

(c) a copy of the Verification Statement executed by Applicants' representative and received on October 3, 2003;

(d) a copy of the Petition for Revival executed by Applicants' representative and received on October 3, 2003;

(e) a copy of the Response to Notice of Defective Response executed by Applicants' representative and received on October 3, 2003;

(f) a copy of the Preliminary Amendment executed by Applicants' representative and received on October 3, 2003.

(2) A Supplemental Preliminary Amendment and Substitute Sequence Listing to address the concerns set forth in the Decision.

Applicants respectfully submit that all of the requirements for the Notification and in response to the Decision have now been met. Early consideration and prompt allowance of the pending claims are respectfully requested.

Respectfully Submitted,

Laura A. Donnelly

Laura A. Donnelly
Registration No. 38,435

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(732) 524-1729 (direct)
(732) 524-2134 (facsimile)

Dated: June 28, 2004

Enclosures:
Decision on Petition Mailed April 27, 2004
Copy of October 3, 2003 Filing, Including Diskette

27 APR 200

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Laura A. Donnelly
Senior Patent Counsel
Johnson & Johnson
One Johnson & Johnson Plaza
New Brunswick, N.J. 08933

RECEIVED

APR 30 2004

J&J PAT. DKT. SECTION

In re Application of :
Stefan Leo Jozef MASURE et al. :
Application No.: 09/869,079 :
PCT Application No.: PCT/GB99/04311 : DECISION ON
International Filing Date: 17 December 1999 :
Priority Date: 22 December 1998 : PETITION
Attorney Docket No.: JAB-1458 :
For: HUMAN AKT-3 : UNDER 37 CFR 1.137(b)

Applicant's "Petition for Revival of an International Application for Patent Designating the U.S. Abandoned Unintentionally Under 37 CFR 1.137(b)," filed in the United States Patent and Trademark Office (USPTO) on 05 January 2004 is **DISMISSED**.

BACKGROUND

On 05 December 2001, the USPTO, in the capacity as Designated/Elected Office, mailed out a DO/EO/905 Form ("Notification of Missing Requirements Under 35 U.S.C. 371") for this application. The DO/EO/905 Form stated that the biochemical "Sequence Listing" did not comply with the requirements of 37 CFR 1.822 and/or 1.832. The Form further stated that applicants were required to provide a substitute paper copy of the "Sequence Listing" and an amendment directing its entry into the specification.

On 15 April 2002, Applicant timely (with a three-month extension of time) filed a Response to the DO/EO/905 Form. The Response included a substitute "Sequence Listing" in paper and computer-readable form and an amendment directing entry of the substitute paper "Sequence Listing" into the specification.

On 11 June 2002, the USPTO mailed out a DO/EO/903 Form ("Notice of Acceptance of Application under 35 U.S.C. 371"). However, on 05 December 2002, the USPTO mailed out a communication stating that the DO/EO/903 Form was sent in error and had been withdrawn. The communication was accompanied by a DO/EO/916 Form ("Notification of Defective Response"). The DO/EO/916 Form stated a problem with the "Sequence Listing" and further stated that applicant must provide a substitute copy of the sequence listing and an amendment directing its entry into the specification. The Form set a non-extendable one month period for reply. Therefore applicant was required to send a proper reply that was received by 05 January 2003 in

order to prevent the application from being abandoned.

On 04 December 2003, the USPTO mailed out a DO/EO/909 Form ("Notice of Abandonment"). The DO/EO/909 Form stated that the application is abandoned as to the United States of America because applicant failed to respond to the 05 December 2002 Notification of Defective Response.

On 05 January 2004, the USPTO received a faxed communication from applicant. The unsigned cover page of the faxed communication states that the papers the follow are copies of papers that were deposited with the U.S. Post Office on September 30, 2003. The cover page further states that a copy of a date stamped postcard, dated October 3, 2003, accompanies the papers. In addition, the cover page states, "Not included is a computer copy of the Sequence Listing for obvious reasons." The papers that follow the cover page include, *inter alia*, the petition to revive and a "Response to the Notice of Defective Response" that includes an amendment and a substitute paper copy of the "Sequence Listing." A copy of a postcard receipt inventorying these items is included. However, the copy of the postcard receipt does not bear an official USPTO date stamp.

DISCUSSION

The petition under 37 CFR 1.137(b) faxed to the USPTO on 05 January 2004 will be treated as having been filed for the first time on that date. It will not be treated as having first been filed on 03 October 2003. The cover page of the faxed communication, which appears to request relief under 37 CFR 1.181for an earlier filing date based upon postcard evidence, is not signed by a registered practitioner, and threfore that paper cannto be treated on the merits. *See* 37 CFR 1.33. In addition, it is noted that the copy of the postcard receipt that is included in the faxed transmission does not bear an official USPTO date stamp. Therefore, it cannot serve as *prima facie* evidence of the date of original submission of the papers. *See* MPEP § 503.

A petition to revive an abandoned application under 37 CFR 1.137(b) must be filed without intentional delay from the time the application became abandoned and/or applicant first became aware of the abandoned status of the application. A petition under 37 CFR 1.137(b) must be accompanied by (1) a statement that the entire delay in filing the required reply from the due date for the reply until the filing of a grantable petition was unintentional, (2) a proper reply, (3) the petition fee required by law (37 CFR 1.17(m)), and (4) a terminal disclaimer and fee (if the international application was filed prior to 08 June 1995). A proper reply in this case requires a reply that is fully responsive to the 05 December 2002 Notification of Defective Response.

Applicant has met the first and fourth requirements of 37 CFR 1.137(b). Applicant has stated, "The entire delay in filing the required reply from the due date for the required reply until the filing of a grantable petition under 37 CFR 1.137(b) was unintentional." A terminal disclaimer is not required because the application was filed on or after 08 June 1995.

With respect to the petition fee, the petition to revive includes an authorization to deduct \$110.00 dollars from the deposit account of Applicant's representative. This amount is not sufficient. The petition fee set forth in 37 CFR 1.17(m) for an entity that does not or cannot claim small entity status is \$1,330.00. However, the petition also includes authorization to deduct the deposit account for any additional fee. Therefore, the deposit account has been charged an additional \$1220.00. Accordingly, the applicant has met the third requirement of 37 CFR 1.137(b).

However, the petition cannot be granted because applicant fails to meet the second requirement of 37 CFR 1.137(b). The first reason why the reply is deficient is that it does not include a computer-readable copy of the latest substitute Sequence Listing. 37 CFR 1.825(b) states, "Any amendment to the paper copy of the "Sequence Listing," in accordance with paragraph (a) of this section, must be accompanied by a substitute copy of the computer readable form (§ 1.821(e)) . . . ." The cover page of the faxed communication explicitly states that a computer-readable copy is not being submitted "for obvious reasons." There is no record that a computer-readable copy was submitted on 03 October 2003 and Applicant has not presented *prima facie* evidence that a copy was filed on that date. Even if such evidence was submitted, a replacement computer-readable copy is still required for processing purposes.

The reply is further deficient because it does not address all of the problems identified on the 05 December 2002 Notification of Defective Response and the attachments that were sent along with the Notification. In particular the Comment Sheet from Technology Center 1600 that was attached to the Notification indicates that there are nucleotide sequences in lines 6-8 *and* lines 12-14 on page 25 of the specification that are not included in the Sequence Listing. The amendment submitted on 05 January 2004 includes a replacement paragraph for the paragraph that begins on line 2 of page 25. This replacement paragraph provides "SEQ ID Nos." for the sequences that are in lines 6-8 of page 25 (lines 4-6 of the replacement paragraph, where the paragraph heading is line 1), but does not provide "SEQ ID Nos." for the two sequences that are listed in lines 12-14 of page 25 (lines 8-10 of the replacement paragraph). The "Remarks" section of the amendment does not provide a reason why these two sequences are not added to the Sequence Listing. Because both of these sequences are longer than ten nucleotides, applicant must include them in the Sequence Listing. *See* 37 CFR 1.821(a) & 1.821(c). Similarly, applicant must also include in the Sequence Listing the amino acid sequence in line 36 on page 20 of the current specification. This sequence is also listed on the aforementioned Comment Sheet. The replacement paragraph in the 05 January 2004 amendment for the paragraph beginning on line 16 of page 19 does not provide a "SEQ ID No." for this sequence.

CONCLUSION

The petition under 37 CFR 1.137(b) to revive the application abandoned as to the National Stage in the United States of America is **DISMISSED**.

Applicant may file a request for reconsideration of this decision within a time period of **TWO (2) MONTHS** from the mailing date of this decision. *See* 37 C.F.R. 1.137(e). Any request for reconsideration should include a cover letter entitled "Renewed Petition Under 37 C.F.R. 1.137(b)." No additional petition fee is required for reconsideration. This time period may be extended under 37 C.F.R. 1.136(a). A request for reconsideration should be mailed to:

Mail Stop PCT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 223130-1450

The contents of the letter should be marked to the attention of the Office of PCT Legal administration.

Boris Milef
PCT Legal Examiner
PCT Legal Administration

Ankur Parekh
Detailee
PCT Legal Administration

Telephone: (703) 308-1315
Facsimile: (703) 308-6459

PatentIn Ver. 2.0
MASURE, STEFAN et al.
Appl. No. 09/809,079
Filed: 17-DEC-1999
Data Rec: 28-JUN-2004
Atty. Dkt. No.: JAB 1468
Copy of October 3, 2004 Submission

Docket No. JAB-1458

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	MASURE et al.
Serial No.	:	09/869,079
Filed	:	I.A. 12/17/99
Title	:	HUMAN AKT-3
Art Unit	:	Unassigned
Examiner	:	Unassigned

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on

June 28, 2004
(Date of Deposit)

Laura A. Donnelly
(Name of applicant, assignee, or Registered Representative)

Laura A. Donnelly
(Signature)

June 28, 2004
(Date of Signature)

Mail Stop PCT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
Attention: PCT Legal Administration

SUPPLEMENTAL PRELIMINARY AMENDMENT

Dear Sir:

Prior to examination on the merits, please amend the above-identified application as follows:

Amendments to the Specification

Please replace the paragraph beginning at page 3, line 20, as originally filed with the following amended paragraph:

Figure 1 is an alignment of the deduced amino acid sequences for human Akt-1 (SEQ ID NO: 15), Akt-2 (SEQ ID NO: 16) and Akt-3 (SEQ ID NO: 3). The sequences were aligned using the ClustalW alignment program (EMBL, Heidelberg, Germany). Amino acid residues conserved between all three proteins are included in the black areas. Residues conserved between only two of the sequences are shaded in grey. Amino acid residues are numbered in the right hand column. The conserved Thr and Ser residues that are presumed to be phosphorylated upon activation are marked with an asterisk above the sequence.

Please replace the paragraph beginning at page 19, line 16, as originally filed with the following amended paragraph:

Molecular cloning of human Akt-3.

Using the rat RAC-Pkγ sequence (Konishi et al, 1995; GenBank acc. No. D49836) as a query sequence, a BLAST (Basic Local Alignment Search Tool; Altschul et al., 1990) search was carried out in the WashU Merck expressed sequence tag (EST) database (Lennon et al., 1996) and in the proprietary LifeSeq™ human EST database (Incyte Pharmaceuticals Inc, Palo Alto, CA, USA). Several human EST clones with high similarity to the rat RAC-Pkγ were identified. One EST sequence (Incyte accession number 2573448) derived from a hippocampal cDNA library, contained part of the coding sequence including the putative methionine start codon (ATG) and part of the 5' untranslated region. The start codon was surrounded by a Kozak consensus sequence for translation start and an in-frame stop codon was present at positions –6 to –3. Based on this 239 bp sequence, oligonucleotide sense primers were synthesised for 3' rapid amplification of cDNA ends (3' RACE) experiments: Akt-3spl = 5'-ACC ATT TCT CCA AGT TGG GGG CTC AG-3' (SEQ ID No: 4) and Akt-3sp2 = 5'GGG AGT CAT CAT GAG CGA TGT TAC C-3' (SEQ ID No: 5). 3'RACE experiments were performed on human fetal brain or human cerebellum Marathon-Ready™ cDNA (Clontech Laboratories, Palo Alto, CA,

USA) according to ~~manufacturer=s~~ manufacturers ~~manufacturer=s~~ instructions using Akt-3sp1/race-ap1 as primers in the primary PCR and Akt-3sp2/race-ap2 in the nested PCR. Resulting PCR fragments were cloned and sequenced. This extended the Akt-3 coding sequence by 916 bp, but the novel sequence did not include an in-frame stop codon. A second round of 3' RACE amplification was performed on human brain Marathon-Ready™ cDNA using sense primers based on the sequence obtained in the first round (Akt-3sp3 = 5'CAC TCC AGA ATA TCT GGC ACC AGA GG-3' (SEQ ID No: 6) and Akt-3sp4 = 5' CTA TGG CCG AGC AGT AGA CTG GTG G-3' (SEQ ID No. 7)) in combination with race-ap1 and race-ap2, respectively. The sequence obtained included an in-frame stop codon and the 3' untranslated sequence up to the poly(A) tail. Antisense primers were designed based on the 3' untranslated region (Akt-3ap4 = 5'-TGC CCC TGC TAT GTG TAA GAG CTA GG-3' (SEQ ID No: 8)) and Akt-3ap5 = 5' AAG AGC TAG GAC TGG TGA TGT CCA GG-3' (SEQ ID No: 9)) and the complete Akt-3 coding sequence was amplified from human hippocampal cDNA using Akt-3sp1/Akt-3ap4 (primary PCR) and Akt-3sp2/Akt-3ap5 (nested PCR) as primers. The resulting 1200 bp PCR fragment was then cloned in the TA-cloning vector pCR2.1 (original TA cloning kit, Invitrogen BV, Leek, The Netherlands) and the inserts of several clones were completely sequenced. One clone containing an insert with the confirmed sequence (hAkt-3/pCR2.1) was used for subsequent subcloning to the mammalian expression vector pcDNA-3 (Invitrogen), yielding construct hAkt-3/pcDNA-3. In order to make a construct coding for a COOH-terminal tagged Akt-3 protein, a fragment of 553 bp was amplified from plasmid Akt-3/pcDNA-3 using an antisense primer incorporating a XhoI restriction site and the sequence coding for a hemagglutinin (HA) tag (YPYDVPDYA) (SEQ ID NO: 13) after amino acid 479 of the Akt-3 sequence. This fragment was recloned into plasmid hAkt-3/pcDNA-3 using BstEII and XhoI restriction sites yielding construct HA-hAkt-3/pcDNA-3.

Please replace the paragraph beginning at page 25, line 3, as originally filed with the following amended paragraph:

Reverse transcription (RT)-PCR analysis

Oligonucleotide primers were designed for the specific PCR amplification of a fragment from Akt-3. These primers were Akt-3sp2 = 5' –GGG AGT CAT CAT GAG CGA TGT TAC C-3' (SEQ ID No: 5) (sense primer) and Akt-3ap1 = 5' – GGG TTG TAG AGG CAT CCA TCT

CTT CC – 3' (SEQ ID No: 11) (antisense primer), yielding a 425 bp product. PCR amplifications for human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) were performed on the same cDNA samples as positive controls using G3PDH primers 5' – TGA AGG TCG GAG TCA ACG GAT TTG GT-3' (sense primer) (SEQ ID NO: 10) and 5' –CAT GTG GGC CAT GAG GTC CAC CAC-3' (antisense primer) (SEQ ID NO: 14), yielding a 1000 bp fragment. These primers were used for PCR amplifications on Multiple Tissue cDNA panels (Clontech Laboratories) and on cDNA prepared from tumor cell lines. For the preparation of tumor cell cDNA, cells were homogenised and total RNA prepared using the RNeasy Mini kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions. 1 Fg of total RNA was reverse transcribed using oligo(dT)$_{15}$ as a primer and 50 U of Expand™ Reverse Transcriptase (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instructions. PCR reactions with Akt-3-specific or G3PDH-specific primers were then performed on 1 Fl of cDNA. Images of the ethidium bromide stained gels were obtained using the Eagle Eye II Video system (Stratagene, La Jolla, CA, USA) and PCR bands analysed using the EagleSight software.

Please replace the paragraph beginning at page 27, line 20, as originally filed with the following amended paragraph:

The predicted Akt-3 (Figure1) protein shows significant similarity with Akt-1 (Jones et al, 1991; 83.6% identity; 87.8% similarity) and with Akt-2 (Cheng et al., 1992; 78% identity; 84.3% similarity).The COOH-terminal `tail= has been observed in both human and rat Akt-1 and Akt-2 proteins, but it is apparently truncated in the only other reported Akt-3 sequence (rat Akt-3, Konishi et al., 1995; accession number D49836). 3'RACE experiments performed on human cDNAs derived from different tissues did not yield evidence for the existence of a shorter form of Akt-3 that would be analogous to the rat Akt-3 (data not shown). The tail in human Akt-3 comprises 28 amino acid residues (YDEDGMDCMDNERRPHFPQFSYSASGRE) **(SEQ ID NO: 12)** that replace 3 amino acid residues in the rat sequence (CPL). The tail in human Akt-3 contains a serine residue at position 472 (shown in bold) that

corresponds to Ser^{473} in Akt-1 or Ser^{474} in Akt-2. Phosphorylation of Ser^{473} and Ser^{474} has previously been implicated in the activation of Akt-1 and Akt-2, respectively (Alessi et al., 1996; Meier et al.,1997). Thr^{308} (in the kinase domain) has also been implicated in the activation of Akt-1 and this residue is also conserved in human Akt-3 (Thr^{305}).

Please replace the paragraph beginning at page 32, line 8, as originally filed with the following amended paragraph:

The sequence which has been identified represents the human homologue of Akt-3. This assignment is based on the >99% identity between the rat and human Akt-3 protein sequences. With the exception of the COOH-terminal tail seen in human Akt-3, there are only 2 amino acid differences (Gly^{10} and Ala^{396} in human Akt-3) between the rat and human Akt-3 proteins. Alignment of all the previously described Akt sequences demonstrates that Gly^{10} and Ala^{396} in the human protein correspond to Gly and Ala residues respectively in the Akt-1 and Akt-2 sequences identified from other species. Further evidence that we have identified the Akt-3 isoform comes from the presence of isotype-specific sequences represented by human Akt-3 residues 47-49 (LPY), 118-122 **of SEQ ID NO: 3** (NCSPT) and 139-141 (HHK). For each isotype, these sequences are conserved between species, but differ between the isotypes.

Please replace pages 45-51 as filed with the Sequence Listing attached hereto.

REMARKS

In response to the Decision on Petition Under 37 C.F.R. § 1.137(b), dated April 27, 2004 ("Decision"), enclosed herewith is a computer readable Sequence Listing, a paper copy and the required Verification Statement Under 37 C.F.R. 1.821(f). This response is also accompanied by a Request for Reconsideration of the Decision. Applicants respectfully submit that all of the requirements for submission of a Sequence Listing have now been met. Early consideration and prompt allowance of the pending claims are respectfully requested.

Respectfully Submitted,

Laura A. Donnelly

Laura A. Donnelly
Registration No. 38,435

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(732) 524-1729 (direct)
(732) 524-2134 (facsimile)

Dated:

Enclosures:
- Computer Readable Sequence Listing
- Paper Copy
- Verification Statement Under 37 C.F.R. 1.821(f)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty. Docket No: JAB-1458

In re patent application of

MASURE, STEFAN *et al.*

Serial No. 09/869,079

Filed: December 17, 1999

For: HUMAN AKT-3

STATEMENT TO SUPPORT FILING AND SUBMISSION IN ACCORDANCE WITH 37 C.F.R. §§ 1.821-1.825

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Mail Stop SEQUENCE

Sir:

In connection with a Sequence Listing submitted concurrently herewith, the undersigned hereby states that:

1. the submission, filed herewith in accordance with 37 C.F.R. § 1.821(g), does not include new matter;

2. the content of the attached paper copy and the attached computer readable copy of the Sequence Listing, submitted in accordance with 37 C.F.R. § 1.821(c) and (e), respectively, are the same.

Respectfully submitted,

June 24, 2004
Date

James A. Coburn

HARBOR CONSULTING IP SERVICES, INC.
1500A Lafayette Road, #262
Portsmouth, N.H.
800-318-3021

SEQUENCE LISTING

<110> MASURE, STEFAN
RICHARDSON, ALAN

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<140> 09/869,079
<141> 1999-12-17

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Asp Gln Arg Thr His Phe Pro Gln Phe Ser Tyr Ser Ala Ser Ile Arg
465 470 475 480

Glu

HUMAN AKT-3

FIELD OF THE INVENTION[1]

The present invention is concerned with cloning and expression of a new human serine/threonine kinase termed ~~AAkt~~[2]"Akt[3]-3≅[4]"[5] and, in particular, with nucleic acid molecules encoding the Akt-3 protein, the protein itself and compounds which can be used to inhibit cell survival.

BACKGROUND OF THE INVENTION[6].

A characteristic feature of many cancer cells is their ability to grow independently of adhesion. In contrast, when untransformed endothelial cells are prevented from adhering to the extracellular matrix (ECM), they undergo apoptosis (Frisch & Francis, 1994; Meredith et al, 1993). The process by which normally adherent cells are triggered to undergo apoptosis when they are unable to adhere to ECM has been termed ~~Aanoikis≅~~[7]"anoikis"[8] (Frisch & Ruoslahti, 1997) and is an example of the effect on a cell of removal of a survival factor. Changes in signalling by adhesion molecules can lead to resistance to anoikis (Frisch & Ruoslahti, 1997) and this may contribute to the mechanism whereby cancer cells that grow independently of adhesion are able to avoid anoikis.

Akt (also known as protein kinase B (PKB) or ~~Arelated~~[9]"related[10] to A and C protein kinase≅[11]"[12] (RAC-PK)) is a serine/threonine kinase that has been implicated in regulating cell survival (Khwaja et al., 1997; Dudek et al., 1997; Kauffmann-Zeh et al., 1997; Kennedy et al., 1997; Datta et al., 1997; Marte & Downward, 1997). Akt can inhibit apoptosis induced by detachment from ECM (~~anoikis; –~~[13]Khwaja et al., 1997), as well as by
survival factor withdrawal (Kennedy et al., 1997;

Ahmed et al., 1997; Dudek et al., 1997; Kauffman-Zeh et al., 1997; Philpott et al., 1997; Crowder & Freeman, 1998; Eves et al., 1998) or irradiation (Kulik et al., 1997).

Akt comprises an NH_2-terminal pleckstrin homology (PH) domain involved in lipid binding, a kinase domain and a COOH-terminal ~~Atail=~~[14]"tail"[15]. Akt is thought to be activated by recruitment to the plasma membrane and subsequent phosphorylation by two upstream kinases, PDK-1 and PDK-2 (reviewed in Coffer et al, 1998; Alessi & Cohen, 1998). The binding of 3-phosphoinositides, generated by phosphatidylinositol 3-kinase (PI 3-kinase), to the PH domain of Akt is believed to promote translocation to the plasma membrane and to facilitate phosphorylation of Akt-1 by PDK-1 at Thr^{308} (Alessi et al., 1996; Alessi et al., 1997; Stephens et al., 1998) or of Akt-2 at Thr^{309} (Meier et al., 1997). In addition to phosphorylation of Thr^{308}, full activation requires phosphorylation of the COOH tail at Ser^{473} in Akt-1 (Alessi et al, 1996) or at Ser^{474} in Akt-2 (Meier et al., 1997). The enzyme responsible for phosphorylation of Ser^{473}/Ser^{474} was originally named PDK-2 but recently the integrin-linked kinase, ILK (Delcommenne et al., 1998) has emerged as a candidate for this function.

Two human isoforms of Akt have been described to date, Akt-1 and Akt-2 (Coffer & Woodgett, 1991; Jones et al., 1991; Cheng et al., 1992). A third isoform, here referred to as Akt-3, has been described in the rat (Konishi et al., 1995). Since this rat Akt-3 possesses an apparently truncated tail and thereby lacks Ser^{473}, its regulation may differ from that of Akt-1 and Akt-2. Both Akt-1 and Akt-2 are expressed widely, although the expression of Akt-2 is most prominent in insulin-responsive tissues, such as liver and skeletal muscle (Konishi et al., 1994; Altomare et al., 1995). Akt-1 and Akt-2 are activated by insulin in rat adipocytes,

hepatocytes and skeletal muscle. In contrast, Akt-3 does not appear to be strongly activated by insulin in these tissues (Walker et al., 1998). The role of the various Akt isoforms in insulin signalling may limit the utility of compounds that inhibit Akt-1 or Akt-2 activity as such agents may induce symptoms observed in patients with diabetes. We hypothesized that this problem may be avoided by using selective inhibitors of Akt-3 and this prompted us to identify the human analogue of rat Akt-3.

BRIEF DESCRIPTION OF THE FIGURES[16]

The present invention may be more clearly understood with reference to the following example which is purely exemplary and the accompanying drawings wherein:[17]

Figure 1 is an alignment of the deduced amino acid sequences for human Akt-1, Akt-2 and Akt-3. The sequences were aligned using the ClustalW alignment program (EMBL, Heidelberg, Germany). Amino acid residues conserved between all three proteins are included in the black areas. Residues conserved between only two of the sequences are shaded in grey. Amino acid residues are numbered in the right hand column. The conserved Thr and Ser residues that are presumed to be phosphorylated upon activation are marked with an asterisk above the sequence.[18]

Figure 2 is an illustration of phosphorylation of histone H2B by Akt-3 variants. (A) Akt-3 was expressed as a GST fusion protein in *E. Coli*. To assess hAkt-3 activity, Histone H2B was incubated with GST-Akt-3 and GST-Akt-3 variants for the indicated time and the extent of phosphorylation assessed after SDS-PAGE. The variants of Akt-3 are designated: W.T., wild type; T305D, Thr^{305} mutated to Asp; S472D, Ser^{472} mutated to Asp; T305D,S472D, both Thr^{305} and Ser^{472} mutated to Asp. No significant phosphorylation was observed when GST

was used in place of GST-Akt. The results are the mean (+s.e.m.; n = 3 to 6) and are expressed relative to the extent of phosphorylation of H2B catalysed by T305D, S472D hAkt-3 after 45 minutes.[19] (B) HEK-293 cells were transfected with either vector (lanes 1 & 2) or Akt-3 (lanes 3 and 4) AKt-3T305A (lanes 5 & 6) or Akt-3 S472A (lanes 7 and 8) and either treated with buffer (lanes 1, 3 5 and 7) or IGF-1 (50 ng/ml; lanes 2, 4, 6 and 8). Akt-3 was immunoprecipitated with antibody 3F10 (anti-HA tag). Samples were analysed by blotting for the HA-tag (upper panel) or with a phosphospecific antibody which recognises phosphorylated ser^{472} (lower panel). (C) Akt activity in HA-immuno-precipitates from samples prepared as described above was assessed by measuring phosphorylation of a peptide substrate (Crosstide). The results are expressed as the increase in activity compared to unstimulated cells transfected with empty vector (mean + s.e.m., n=7).[20]

Figure 3 is an illustration of inhibition of Akt-3 by staurosporine and R0 31-8220. Histone H2B was treated with Akt-3 (T305D,S472D variant) in the presence of the indicated concentrations of either staurosporine or R0 31-8220. After 30 minutes, the reaction was terminated and the extent of H2B phosphorylation quantified on a phosphorimager following SDS-PAGE. The results (mean +s.e.m., n=3) are expressed as relative to (%) the phosphorylation observed in the presence of solvent (control, "C").[21]

Figure 4 is an illustration of chromosomal localisation of human Akt-3. Diagram of FISH mapping results of Akt-3. Each dot represents the double FISH signals detected on human chromosome 1, region q43-q44.[22]

Figure 5 is an illustration of expression of Akt-3 in different human tissues. (A) Northern blot analysis of

tissue expression of Akt-3. The expression of hAkt-3 mRNA in different human tissues was assessed using a probe corresponding to the 3' untranslated region of hAkt-3 to analyse a blot of human polyA$^+$ RNA (AMultiple Tissue Northern≅). Human β-actin was used as a control to confirm equal loading of the lanes (data not shown). (B) and (C) RT-PCR analysis of tissue expression of Akt-3. RT-PCR analyses were performed on cDNA from different human tissues (B) and from different tumor cell lines (C) using primers specific for human Akt-3 or G3PDH (control) for the indicated number of PCR cycles. Bands of the expected size (425 bp for Akt-3 and 1 kb for G3PDH) are visible on the gels. The images from the ethidium bromide stained 1.2% agarose gels were inverted for clarity using the EagleSight software (Stratagene). The results from similar PCR reactions performed for 25, 30 or 35 cycles are not shown but indicated that the results from this figure are in the linear range of amplification. Caco-2 = colorectal adenocarcinoma; T-84 = colorectal carcinoma; MCF-7 = breast adenocarcinoma; T-47D = breast ductal gland carcinoma; HT1080 = bone fibrosarcoma; SaOS-2 = osteosarcoma; SK-N-MC = neuroblastoma; HepG2 = hepatoblastoma; JURKAT = T-cell leukemia.[23]

Figure 6 is an illustration of the results obtained by scintillation counting in a scintillation proximity assay to identify agents that modulate the activity of Akt-3 activity.[24]

Figure 7 is an illustration of the results obtained from an Akt-3 filter assay to identify agents that modulate activity of Akt-3.[25]

DETAILED DESCRIPTION OF THE INVENTION[26]

The present inventors have now identified and characterised a nucleic acid molecule that encodes the

human isoform of Akt-3. Significantly, human Akt-3 possesses a COOH-terminal tail that contains an amino acid residue analogous to Ser^{473}/Ser^{474} previously implicated in the activation of Akt-1/Akt-2, but absent in the rat Akt-3 protein.

Therefore, there is provided by a first aspect of the present invention a nucleic acid molecule encoding human Akt-3 or a functional equivalent, derivative or bioprecursor thereof, comprising the amino acid sequence illustrated in Figure ~~2.~~[27]1 (and as SEQ ID NO:3).[28]

Preferably, the molecule is a DNA molecule and even more preferably a cDNA molecule, and even more preferably comprises the sequence of nucleotides ~~illustrated~~[29]provided[30] in ~~Figure~~[31]SEQ ID NO:[32]1. Also provided by this aspect of the invention is a nucleic acid molecule capable of hybridising to the molecule according to the invention under high stringency conditions.

Stringency of hybridisation as used herein refers to conditions under which polynucleic acids are stable. The stability of hybrids is reflected in the melting temperature (Tm) of the hybrids. Tm can be approximated by the formula:

81.5~~EC~~[33]°C[34]+16.6($\log_{10}[Na^+]$+0.41 (%G&C)-600/1

wherein 1 is the length of the hybrids in nucleotides. Tm decreases approximately by 1-1.5~~EC~~[35]°C[36] with every 1% decrease in sequence homology.

The term ~~Astringency=~~[37]"stringency"[38] refers to the hybridisation conditions wherein a single-stranded nucleic acid joins with a complementary strand when the purine or pyrimidine bases therein pair with their corresponding base by hydrogen bonding. High

stringency conditions favour homologous base pairing whereas low stringency conditions favour non-homologous base pairing.

~~ALow~~[39]"Low[40] stringency≅[41]"[42] conditions comprise, for example, a temperature of about 37~~EC~~[43]°C[44] or less, a formamide concentration of less than about 50%, and a moderate to low salt (SSC) concentration; or, alternatively, a temperature of about 50~~EC~~[45]°C[46] or less, and a moderate to high salt (SSPE) concentration, for example 1M NaCl.

~~AHigh~~[47]"High[48] stringency≅[49]"[50] conditions comprise, for example, a temperature of about 42~~EC~~[51]°C[52] or less, a formamide concentration of less than about 20%, and a low salt (SSC) concentration; or, alternatively, a temperature of about 65~~EC~~[53]°C[54], or less, and a low salt (SSPE) concentration. For example, high stringency conditions comprise hybridization in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65~~EC~~[55]°C[56] (Ausubel, F.M. *et al.* Current Protocols in Molecular Biology, Vol. I, 1989; Green Inc. New York, at 2.10.3).

~~ASSC≅~~[57]"SSC"[58] comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0.

~~ASSPE≅~~[59]"SSPE"[60] comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl, 10mM NaH_2PO4 and 1 mM EDTA, pH 7.4.

The nucleic acid capable of hybridising to nucleic acid molecules according to the invention will generally be at least 85%, preferably at least 90% and even more preferably at least 95% homologous to the nucleotide sequences according to the invention.

The DNA molecules according to the invention may, advantageously, be included in a suitable expression vector to express polypeptides encoded therefrom in a suitable host.

The present invention also comprises within its scope proteins or polypeptides encoded by the nucleic acid molecules according to the invention or a functional equivalent, derivative or bioprecursor thereof.

An expression vector according to the invention includes a vector having a nucleic acid according to the invention operably linked to regulatory sequences, such as promoter regions, that are capable of effecting expression of said DNA fragments. The term ~~Aoperably~~[61] "operably[62] linked~~≅~~[63]"[64] refers to a juxta position wherein the components described are in a relationship permitting them to function in their intended manner. Such vectors may be transformed into a suitable host cell to provide for expression of a polypeptide according to the invention. Thus, in a further

aspect, the invention provides a process for preparing polypeptides according to the invention which comprises cultivating a host cell, transformed or transfected with an expression vector as described above under conditions to provide for expression by the vector of a coding sequence encoding the polypeptides, and recovering the expressed polypeptides.

The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of said nucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable markers, such as, for example, ampicillin resistance.

Regulatory elements required for expression include

promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector may include a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG. Similarly, a eukaryotic expression vector may include a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art.

A nucleic acid molecule according to the invention may be inserted into the vectors described in an antisense orientation in order to provide for the production of antisense RNA. Antisense RNA or other antisense nucleic acids may be produced by synthetic means.

In accordance with the present invention, a defined nucleic acid includes not only the identical nucleic acid but also any minor base variations including in particular, substitutions in bases which result in a synonymous codon (a different codon specifying the same amino acid residue) due to the degenerate code in conservative amino acid substitutions. The term ~~Anucleic~~[65]"nucleic[66] acid sequence≅[67]"[68] also includes the complementary sequence to any single stranded sequence given regarding base variations.

The present invention also advantageously provides nucleic acid sequences of at least approximately 10 contiguous nucleotides of a nucleic acid according to the invention and preferably from 10 to 120, and even more preferably from 10 to approximately 50 nucleotides. These sequences may, advantageously be used as probes or primers to initiate replication, or the like. Such nucleic acid sequences may be produced

according to techniques well known in the art, such as by recombinant or synthetic means. They may also be used in diagnostic kits or the like for detecting the presence of a nucleic acid according to the invention. These tests generally comprise contacting the probe with the sample under hybridising conditions and detecting for the presence of any duplex or triplex formation between the probe and any nucleic acid in the sample.

According to the present invention these probes may be anchored to a solid support. Preferably, they are present on an array so that multiple probes can simultaneously hybridize to a single biological sample. The probes can be spotted onto the array or synthesised *in situ* on the array. (See Lockhart *et al.*, Nature Biotechnology, vol. 14, December 1996 ~~AExpression~~[69]"Expression[70] monitoring by hybridisation to high density oligonucleotide arrays≅[71]")[72]. A single array can contain more than 100, 500 or even 1,000 different probes in discrete locations.

Advantageously, the nucleic acid sequences, according to the invention may be produced using such recombinant or synthetic means, such as for example using PCR cloning mechanisms which generally involve making a pair of primers, which may be from approximately 10 to 50 nucleotides to a region of the gene which is desired to be cloned, bringing the primers into contact with mRNA, cDNA, or genomic DNA from a human cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolating the amplified region or fragment and recovering the amplified DNA. Generally, such techniques as defined herein are well known in the art, such as described in Sambrook *et al* (Molecular Cloning: a Laboratory Manual, 1989).

The nucleic acids or oligonucleotides according to the invention may carry a revealing label. Suitable labels include radioisotopes such as ^{32}P or ^{35}S, enzyme labels or other protein labels such as biotin or fluorescent markers. Such labels may be added to the nucleic acids or oligonucleotides of the invention and may be detected using known techniques *per se*.

A further aspect of the invention comprises human Akt-3 or a functional equivalent, derivative or bioprecursor thereof, comprising an amino acid sequence as illustrated in Figure ~~2.~~[73] 1.[74]

The polypeptide designated human Akt-3 according to the invention includes all possible amino acid variants encoded by the nucleic acid molecule according to the invention including a polypeptide encoded by said molecule and having conservative amino acid changes. Polypeptides according to the invention further include variants of such sequences, including naturally occurring allelic variants which are substantially homologous to said polypeptides. In this context, substantial homology is regarded as a sequence which has at least 90% amino acid homology with the polypeptides encoded by the nucleic acid molecules according to the invention and even more preferably at least 95% amino acid homology.

The nucleic acid molecule or the human Akt-3 according to the invention may, advantageously, be used as a medicament or in the preparation of a medicament, for treating disease associated with Akt-3 activity such as, cancer or the like.

Advantageously, the nucleic acid molecule or the polypeptide according to the invention may be provided in a pharmaceutical composition together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

The present invention is further directed to inhibiting Akt-3 *in vivo* by the use of antisense technology. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the mature protein sequence, which encodes for the protein of the present invention, is used to design an antisense RNA oligonucleotide of from 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple-helix - see Lee *et al.* Nucl. Acids Res., 6:3073 (1979); Cooney *et al.*, Science, 241:456 (1988); and Dervan *et al.*, Science, 251: 1360 (1991), thereby preventing transcription and the production of Akt-3. The antisense RNA oligonucleotide hybridises to the mRNA *in vivo* and blocks translation of an mRNA molecule into the Akt-3 (antisense - Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1998)).

Alternatively, the oligonucleotide described above can be delivered to cells by procedures in the art such that the anti-sense RNA or DNA may be expressed *in vivo* to inhibit production of Akt-3 in the manner described above.

Antisense constructs to Akt-3, therefore, may inhibit the survival of the cell and prevent further cancer or tumour growth.

According to a further aspect of the invention there is also provided a transgenic cell, tissue or organism comprising a transgene capable of expressing human Akt-3 protein according to the invention. The term ~~Atransgene~~[75]"transgene[76] capable of expression≅[77]"[78] as

used herein means a suitable nucleic acid sequence which leads to expression of human Akt-3 or human proteins having the same function and/or activity. The transgene, may include, for example, genomic nucleic acid isolated from human cells or synthetic nucleic acid, including DNA integrated into the genome or in an extrachromosomal state. Preferably, the transgene comprises the nucleic acid sequence encoding the proteins according to the invention as described herein, or a functional fragment of said nucleic acid. A functional fragment of said nucleic acid should be taken to mean a fragment of the gene comprising said nucleic acid coding for the proteins according to the invention or a functional equivalent, derivative or a non-functional derivative such as a dominant negative mutant, or bioprecursor of said proteins. For example, it would be readily apparent to persons skilled in the art that nucleotide substitutions or deletions may be used using routine techniques, which do not affect the protein sequence encoded by said nucleic acid, or which encode a functional protein according to the invention.

Human Akt-3 protein expressed by said transgenic cell, tissue or organism or a functional equivalent or bioprecursor of said protein also form part of the present invention.

Antibodies to human Akt-3 may, advantageously, be prepared by techniques which are known in the art. For example, polyclonal antibodies may be prepared by inoculating a host animal, such as a mouse, with human Akt-3 according to the invention or an epitope thereof and recovering immune serum. Monoclonal antibodies may be prepared according to known techniques such as described by Kohler R. and Milstein C., Nature (1975) 256, 495-497.

Antibodies according to the invention may also be used

in a method of detecting for the presence of human Akt-3 according to the invention, which method comprises reacting the antibody with a sample and identifying any protein bound to said antibody. A kit may also be provided for performing said method which comprises an antibody according to the invention and means for reacting the antibody with said sample.

Proteins which interact with the polypeptide of the invention may be identified by, for example, investigating protein-protein interactions using the two-hybrid vector system first proposed by Chien *et al* (1991). Proc. Natl. Acad. Sci. USA 88: 9578-9582.

This technique is based on functional reconstitution in vivo of a transcription factor which activates a reporter gene. More particularly the technique comprises providing an appropriate host cell with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA binding domain and an activating domain, expressing in the host cell a first hybrid DNA sequence encoding a first fusion of a fragment or all of a nucleic acid sequence according to the invention and either said DNA binding domain or said activating domain of the transcription factor, expressing in the host at least one second hybrid DNA sequence, such as a library or the like, encoding putative binding proteins to be investigated together with the DNA binding or activating domain of the transcription factor which is not incorporated in the first fusion; detecting any binding of the proteins to be investigated with a protein according to the invention by detecting for the presence of any reporter gene product in the host cell; optionally isolating second hybrid DNA sequences encoding the binding protein.

An example of such a technique utilises the GAL4 protein in yeast. GAL4 is a transcriptional activator

of galactose metabolism in yeast and has a separate domain for binding to activators upstream of the galactose metabolising genes as well as a protein binding domain. Nucleotide vectors may be constructed, one of which comprises the nucleotide residues encoding the DNA binding domain of GAL4. These binding domain residues may be fused to a known protein encoding sequence, such as for example the nucleic acids according to the invention. The other vector comprises the residues encoding the protein binding domain of GAL4. These residues are fused to residues encoding a test protein. Any interaction between polypeptides encoded by the nucleic acid according to the invention and the protein to be tested leads to transcriptional activation of a reporter molecule in a GAL-4 transcription deficient yeast cell into which the vectors have been transformed. Preferably, a reporter molecule such as β-galactosidase is activated upon restoration of transcription of the yeast galactose metabolism genes.

A further aspect of the invention provides a method of identifying compounds which selectively inhibit human Akt-3 mediated promotion of cell survival said method comprising i) providing a cell transformed with an expression vector activating the Akt-3 pathway which cell survives in the presence or absence of a survival factor compared to a control cell which has not been transformed with said vector and will die in the absence of said survival factor ii) contacting said cells with a test compound following removal of said cells from said survival factors, wherein death of said transformed cell is indicative of selective inhibition of said compound on the survival promoting human Akt-3 pathway.

Alternatively, the survival promoting activity of Akt-3 could be assessed by i) providing a cell transformed with an expression vector activating the Akt-3 pathway

in addition to a control cell which has not been transformed with said vector, ii) contacting each of said cells with a death inducing agent, whereby death of said control cell and survival of said transformed cell is indicative of the survival promoting activity of the activated Akt-3 pathway, iii) subsequently contacting said transformed cell without removal of said death inducing agent, with a test compound, wherein death of said cell is indicative of selective inhibition of said compound on the survival promoting human Akt-3 pathway.

In a further aspect the present invention provides methods to identify agents that affect the activity of the human Akt-3 protein, comprising contacting said protein with a substrate, regulatory molecule or surrogate thereof and monitoring the interaction with the test substance using standard phosphorylation or binding assays well known in the art.

Compounds which are identified according to this aspect of the invention in addition to antibodies to the human Akt-3 may, advantageously, be utilised as a medicament or alternatively in the preparation of a medicament for treating diseases associated with expression of human Akt-3 protein according to the invention.

A further aspect of the invention provides a pharmaceutical composition comprising any of a compound, an antisense molecule or an antibody according to the invention together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

The antisense molecules or indeed the compounds identified as agonists or antagonists of the nucleic acids or polypeptides according to the invention may be used in the form of a pharmaceutical composition,

which may be prepared according to procedures well known in the art. Preferred compositions include a pharmaceutically acceptable vehicle or diluent or excipient, such as for example, a physiological saline solution. Other pharmaceutically acceptable carriers including other non-toxic salts, sterile water or the like may also be used. A suitable buffer may also be present allowing the compositions to be lyophilized and stored in sterile conditions prior to reconstitution by the addition of sterile water for subsequent administration. Incorporation of the polypeptides of the invention into a solid or semi-solid biologically compatible matrix may be carried out which can be implanted into tissues requiring treatment.

The carrier can also contain other pharmaceutically acceptable excipients for modifying other conditions such as pH, osmolarity, viscosity, sterility, lipophilicity, solubility or the like.

Pharmaceutically acceptable excipients which permit sustained or delayed release following administration may also be included.

The polypeptides, the nucleic acid molecules or compounds according to the invention may be administered orally. In this embodiment they may be encapsulated and combined with suitable carriers in solid dosage forms which would be well known to those skilled in the art.

As would be well known to those of skill in the art, the specific dosage regime may be calculated according to the body surface area of the patient or the volume of body space to be occupied, dependent upon the particular route of administration to be used. The amount of the composition actually administered will, however, be determined by a medical practitioner,

based on the circumstances pertaining to the disorder to be treated, such as the severity of the symptoms, the composition to be administered, the age, weight, and response of the individual patient and the chosen route of administration.

~~The present invention may be more clearly understood with reference to the following example which is purely exemplary and the accompanying drawings wherein:~~[79]
~~Figure 1 is an illustration of the cDNA sequence and deduced amino acid sequence of human Akt-3. The Akt-3 coding sequence and parts of the 5' and 3' untranslated regions are shown and numbered in the left hand column. The deduced amino acid sequence of the Akt-3 protein is shown above the corresponding DNA sequence and is numbered in the right hand column. The two amino acid residues that are presumed to be phosphorylated upon activation of Akt-3 (Thr^{305} and Ser^{472}) are in bold and marked with an asterisk. The COOH-terminal part of the human Akt-3 protein that differs with the rat homologue is underlined.~~[80]

~~Figure 2 is an alignment of the deduced amino acid sequences for human Akt-1, Akt-2 and Akt-3. The sequences were aligned using the ClustalW alignment program (EMBL, Heidelberg, Germany). Amino acid residues conserved between all three proteins are included in the black areas. Residues conserved between only two of the sequences are shaded in grey. Amino acid residues are numbered in the right hand column. The conserved Thr and Ser residues that are presumed to be phosphorylated upon activation are marked with an asterisk above the sequence.~~[81]

~~Figure 3 is an illustration of phosphorylation of histone H2B by Akt-3 variants. (A) Akt-3 was expressed as a GST fusion protein in *E. Coli*. To assess hAkt-3 activity, Histone H2B was incubated with GST-Akt-3 and~~

~~GST-Akt-3 variants for the indicated time and the extent of phosphorylation assessed after SDS-PAGE. The variants of Akt-3 are designated: W.T., wild type; T305D, Thr^{305} mutated to Asp; S472D, Ser^{472} mutated to Asp; T305D,S472D, both Thr^{305} and Ser^{472} mutated to Asp.~~[82] ~~No significant phosphorylation was observed when GST was used in place of GST-Akt. The results are the mean (±s.e.m.; n = 3 to 6) and are expressed relative to the extent of phosphorylation of H2B catalysed by T305D, S472D hAkt-3 after 45 minutes. *Insert*, The purity of the purified GST (lane1), wild-type Akt-3 (lane 2), T305D Akt-3 (lane 3), S472D Akt-3 (lane 4) or T305D/S472D Akt-3 (lane 5) was assessed by SDS-PAGE and by Coomassie blue staining. (B) HEK-293 cells were transfected with either vector (lanes 1 & 2) or Akt-3 (lanes 3 and 4) AKt-3T305A (lanes 5 & 6) or Akt-3 S472A (lanes 7 and 8) and either treated with buffer (lanes 1, 3 5 and 7) or IGF-1 (50 ng/ml; lanes 2, 4, 6 and 8). Akt-3 was immunoprecipitated with antibody 3F10 (anti-HA tag). Samples were analysed by blotting for the HA-tag (upper panel) or with a phosphospecific antibody which recognises phosphorylated ser^{472} (lower panel). (C) Akt activity in HA-immuno-precipitates from samples prepared as described above was assessed by measuring phosphorylation of a peptide substrate (Crosstide). The results are expressed as the increase in activity compared to unstimulated cells transfected with empty vector (mean ± s.e.m., n=7).~~[83]

~~Figure 4 is an illustration of inhibition of Akt-3 by staurosporine and R0-31-8220. Histone H2B was treated with Akt-3 (T305D,S472D variant) in the presence of the indicated concentrations of either staurosporine or R0-31-8220. After 30 minutes, the reaction was terminated and the extent of H2B phosphorylation quantified on a phosphorimager following SDS-PAGE. The results (mean ±s.e.m., n=3) are expressed as relative to (%) the phosphorylation observed in the presence of~~

~~solvent (control, AC≅).~~[84]

~~Figure 5 is an illustration of chromosomal localisation of human Akt-3. (A)Diagram of FISH mapping results of Akt-3. Each dot represents the double FISH signals detected on human chromosome 1, region q43 - q44. (B)Example of FISH mapping of Akt-3. The left panel shows the FISH signals on chromosome 1. The right panel shows the same mitotic figure stained with 4',6- diamidino-2-phenylindole to identify chromosome 1.~~[85]

~~Figure 6 is an illustration of expression of Akt-3 in different human tissues. (A) Northern blot analysis of tissue expression of Akt-3. The expression of hAkt-3 mRNA in different human tissues was assessed using a probe corresponding to the 3' untranslated region of hAkt-3 to analyse a blot of human polyA+ RNA (AMultiple Tissue Northern≅). Human β-actin was used as a control to confirm equal loading of the lanes (data not shown). (B) and (C) RT-PCR analysis of tissue expression of Akt-3. RT-PCR analyses were performed on cDNA from different human tissues (B) and from different tumor cell lines (C) using primers specific for human Akt-3 or G3PDH (control) for the indicated number of PCR cycles. Bands of the expected size (425 bp for Akt-3 and 1 kb for G3PDH) are visible on the gels. The images from the ethidium bromide stained 1.2% agarose gels were inverted for clarity using the EagleSight software (Stratagene). The results from similar PCR reactions performed for 25, 30 or 35 cycles are not shown but indicated that the results from this figure are in the linear range of amplification. Caco-2 = colorectal adenocarcinoma; T-84 = colorectal carcinoma; MCF-7 = breast adenocarcinoma; T-47D = breast ductal gland carcinoma; HT1080 = bone fibrosarcoma; SaOS-2 = osteosarcoma; SK-N-MC = neuroblastoma; HepG2 = hepatoblastoma; JURKAT = T-cell leukemia.~~[86]

~~Figure 7 is an illustration of the results obtained by scintillation counting in a scintillation proximity assay to identify agents that modulate the activity of Akt 3 activity.~~[87]

~~Figure 8 is an illustration of the results obtained from an Akt 3 filter assay to identify agents that modulate activity of Akt 3.~~[88]

EXAMPLES[89]

MATERIALS AND METHODS

Oligonucleotide synthesis and DNA sequence determination

All primers were obtained from Eurogentec, Seraing, Belgium. Insert-specific sequencing primers (15- and 16-mers) were designed by visual inspection of the DNA sequences. DNA was prepared on Qiagen-tip-20 columns or on Qiaquick spin columns (Qiagen GmbH, Düsseldorf, Germany) and recovered from the spin columns in 30 Fl Tris/EDTA-buffer (10mM TrisHCl pH 7.5, 1 mM EDTA (sodium salt)). Sequencing reactions were performed using BigDye™ Terminator Cycle Sequencing Ready Reaction kits (Perkin Elmer, ABI Division, Foster City, CA, USA) and were run on an Applied Biosystems 377 DNA sequencer (Perkin Elmer, ABI Division, Foster City, CA, USA).

Molecular cloning of human Akt-3.

Using the rat RAC-PKγ sequence (Konishi et al, 1995; GenBank acc. No. D49836) as a query sequence, a BLAST (Basic Local Alignment Search Tool; Altschul et al., 1990) search was carried out in the WashU Merck expressed sequence tag (EST) database (Lennon et al., 1996) and in the proprietary LifeSeq™ human EST database (Incyte Pharmaceuticals Inc, Palo Alto, CA, USA). Several human EST clones with high similarity to the rat RAC-PKγ were identified. One EST sequence

(Incyte accession number 2573448) derived from a hippocampal cDNA library, contained part of the coding sequence including the putative methionine start codon (ATG) and part of the 5' untranslated region. The start codon was surrounded by a Kozak consensus sequence for translation start and an in-frame stop codon was present at positions -6 to -3. Based on this 239 bp sequence, oligonucleotide sense primers were synthesised for 3' rapid amplification of cDNA ends(3' RACE) experiments: Akt-3sp1 = 5'-ACC ATT TCT CCA AGT TGG GGG CTC AG-3' and Akt-3sp2 = 5'GGG AGT CAT CAT GAG CGA TGT TAC C-3'. 3'RACE experiments were performed on human fetal brain or human cerebellum Marathon-Ready™ cDNA (Clontech Laboratories, Palo Alto, CA, USA) according to manufacturer=s instructions using Akt-3sp1/race-ap1 as primers in the primary PCR and Akt-3sp2/race-ap2 in the nested PCR. Resulting PCR fragments were cloned and sequenced. This extended the Akt-3 coding sequence by 916 bp, but the novel sequence did not include an in-frame stop codon. A second round of 3' RACE amplification was performed on human brain Marathon Ready™ cDNA using sense primers based on the sequence obtained in the first round (Akt-3sp3 = 5'CAC TCC AGA ATA TCT GGC ACC AGA GG-3' and Akt-3sp4 = 5'CTA TGG CCG AGC AGT AGA CTG GTG G-3') in combination with race-ap1 and race-ap2, respectively. The sequence obtained included an in-frame stop codon and the 3' untranslated sequence up to the poly(A) tail. Antisense primers were designed based on the 3' untranslated region (Akt-3ap4 = 5'-TGC CCC TGC TAT GTG TAA GAG CTA GG-3'and Akt-3ap5 = 5' AAG AGC TAG GAC TGG TGA TGT CCA GG-3') and the complete Akt-3 coding sequence was amplified from human hippocampal cDNA using Akt-3sp1/Akt-3ap4 (primary PCR) and Akt-3sp2/Akt-3ap5 (nested PCR) as primers. The resulting 1200 bp PCR fragment was then cloned in the TA-cloning vector pCR2.1 (original TA cloning kit, Invitrogen BV, Leek, The Netherlands) and the inserts of several clones were completely sequenced. One

clone containing an insert with the confirmed sequence (hAkt-3/pCR2.1) was used for subsequent subcloning to the mammalian expression vector pcDNA-3 (Invitrogen), yielding construct hAkt-3/pcDNA-3. In order to make a construct coding for a COOH-terminal tagged Akt-3 protein, a fragment of 553 bp was amplified from plasmid Akt-3/pcDNA-3 using an antisense primer incorporating a *Xho*I restriction site and the sequence coding for a hemagglutinin (HA) tag (YPYDVPDYA) after amino acid 479 of the Akt-3 sequence. This fragment was recloned into plasmid hAkt-3/pcDNA-3 using *Bst*EII and *Xho*I restriction sites yielding construct HA-hAkt-3/pcDNA-3.

Constructs and mutants for *E. coli* expression of Akt-3. In order to express the human Akt-3 protein in *E. coli*, the complete Akt-3 coding sequence was amplified from plasmid hAkt-3/pCR2.1 using primers introducing a *Eco*RI restriction site and a *Xho*I restriction site at the 5'and 3' ends, respectively. This PCR fragment was cloned as a *Eco*RI/*Xho*I fragment in vector pGEX-4T-3 (Amersham Pharmacia Biotech, Uppsala, Sweden) yielding construct hAKT-3(WT)/pGEX-4T-3, and the sequence of the insert was confirmed by sequence analysis.

Mutants of this construct were made using the Quickchange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) according to the manufacturer=s instructions. The T305D mutant (construct hAKT-3(T305D)/pGEX-4T-3) was created by mutating ACA at position 923-925 to GAC, resulting in a Thr^{305} to Asp mutation in the resulting protein. The S472D mutant (construct hAKT-3(S472D)/pGEX-4T-3) was created by changing TC at position 1404-1405 to GA using PCR with a long antisense primer incorporating the change, resulting in a Ser^{472} to Asp mutation in the resulting protein. A double mutant was also constructed by site-directed mutagenesis on hAKT-3(S472D)/pGEX-4T-3 and contained both these mutations (construct hAKT-

3(T305D/S472D)/pGEX-4T-3). The inserts of all resulting constructs were confirmed by complete sequence analysis. The fusion proteins resulting from expression of these constructs in *E. coli* contain a GST moiety coupled to the NH_2-terminus of the human Akt-3 sequence.

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Expression in Cos-7 cells and HEK-293 cells

Akt-3 was transiently expressed in Cos-7 by calcium phosphate transfection of the cells with the construct HA-hAkt-3/pcDNA-3. The cells were stimulated with 10 ng/ml IGF-1 for 30 minutes, lysed and Akt-3 immunoprecipitated with mAb 12CA5. Akt-3 activity was assessed as described below.

For expression in HEK-293 cells, cells were transfected with pCDNA-3 Akt-3 constructs as described previously (Alessi et al 1996). After stimulation with IGF, the cells were lysed (Alessi et al 1996) and HA-Akt immunoprecipitated with antibody 3F10 (Roche Molecular Biochemicals). Akt activity was assessed in immune complexes by measuring phosphorylation of a peptide substrate (Crosstide) in the presence of 1 μM PKI (PKA inhibitor) and 1 μM GF 109302X (PKC inhibitor) as described.

Expression and assay of wild-type and mutant Akt-3 in *E. coli.*

The pGEX expression constructs were transformed into *E. coli* strain BL21 DE3 and GST-fusion proteins of wild-type and mutated Akt-3 were purified on glutathione sepharose according to the manufacturer=s instructions (Amersham Pharmacia Biotech, Uppsala, Sweden). The protein eluted from the beads was stored in 50% glycerol at -20~~EC~~[91]°C[92]. Akt activity was assessed by incubating 0.8 Fg of the purified enzyme for 30 minutes at room temperature (unless otherwise indicated) in a buffer containing 10 mM HEPES, 10 mM $MgCl_2$, 1 mM DTT, 0.1 mg/ml histone H2B at pH 7.0, in a total volume of 25Fl and containing 10 FCi [γ-^{32}P]-ATP (6000 Ci/mmol). Initial experiments indicated that the reaction was linear with time for at least 45 minutes. The reaction was stopped by the addition of 25 Fl sample buffer for SDS-PAGE. The results were

quantified on a phosphorimager following SDS-PAGE on a 15% (w/v) acrylamide gel.

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Chromosomal mapping studies

Chromosomal mapping studies were carried out by SeeDNA Biotech Inc, Toronto, Canada using fluorescent *in situ* hybridisation (FISH) analysis essentially as described (Heng et al., 1992; Heng & Tsui, 1993). Briefly, human lymphocytes were cultured at 37~~EC~~[94]°C[95] for 68-72 h before treatment with 0.18 mg/ml 5-bromo-2'-deoxyuridine (BrdU) to synchronize the cell cycle in the cell population. The synchronized cells were washed and recultured at 37~~EC~~[96]°C[97] for 6 h. Cells were harvested and slides were prepared using standard procedures including hypotonic treatment, fixation and air-drying. A cDNA probe for Akt-3 (1.44 kb *Eco*RI fragment of clone hAkt-3/pcDNA-3) was biotinylated and used for FISH detection. Slides were baked at 55~~EC~~[98]°C[99] for 1 h, treated with Rnase and denatured in 70% (v/v) formamide in 2x NaCl/Cit (0.3 M NaCl, 0.03 M disodium citrate, pH 7.0) for 2 min at 70EC followed by dehydration in ethanol. Probes were denatured prior to loading on the denatured chromosomal slides. After overnight hybridisation, slides were washed and FISH signals and the 4',6-diamidiono-2-phenylindole banding pattern were recorded separately on photographic film, and the assignment of the FISH mapping data with chromosomal bands was achieved by superimposition of FISH signals with 4,6-diamidino-2-phenylindole banded chromosomes (Heng & Tsui, 1993).

Northern blot analysis.

Northern blots containing 2 Fg of poly(A)-rich RNA derived from different human tissues (Clontech Laboratories, Palo Alto, CA, USA) were hybridised according to the manufacturer~~=~~[100]'[101]s instructions with a α-^{32}P-dCTP random-priming labelled (HighPrime kit, Boehringer Mannheim) 454 bp *Not*I-*Xba*I Akt-3 fragment (nucleotides 1404 to 1857) corresponding to part of the 3' untranslated sequence.

Reverse transcription (RT)-PCR analysis

Oligonucleotide primers were designed for the specific PCR amplification of a fragment from Akt-3. These primers were Akt-3sp2 = 5'-GGG AGT CAT CAT GAG CGA TGT TAC C-3' (sense primer) and Akt-3ap1 = 5'- GGG TTG TAG AGG CAT CCA TCT CTT CC -3' (antisense primer), yielding a 425 bp product. PCR amplifications for human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) were performed on the same cDNA samples as positive controls using G3PDH primers 5'-TGA AGG TCG GAG TCA ACG GAT TTG GT-3'(sense primer) and 5'-CAT GTG GGC CAT GAG GTC CAC CAC-3' (antisense primer), yielding a 1000 bp fragment. These primers were used for PCR amplifications on Multiple Tissue cDNA panels (Clontech Laboratories) and on cDNA prepared from tumor cell lines. For the preparation of tumor cell cDNA, cells were homogenised and total RNA prepared using the RNeasy Mini kit (Qiagen GmbH, Hilden, Germany) according to manufacturer=[102]'[103]s instructions. 1 Fg of total RNA was reverse transcribed using oligo(dT)$_{15}$ as a primer and 50 U of Expand™ Reverse Transcriptase (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer=[104]'[105]s instructions. PCR reactions with Akt-3-specific or G3PDH-specific primers were then performed on 1 Fl of cDNA. Images of the ethidium bromide stained gels were obtained using the Eagle Eye II Video system (Stratagene, La Jolla, CA, USA) and PCR bands analysed using the EagleSight software.

Assays to identify agents that modulate the activity of Akt-3

To identify agents that modulate the activity of Akt-3, SPA (scintillation proximity assay) and filter assays for Akt-3 activity were developed.

SPA assays were performed at 25~~EC~~[106]°C[107] for 3 hrs in the presence of 25mM Hepes, pH 7.0, containing 15 mM

$MgCl_2$, 1 mM DTT. Each assay was performed in a 100 Fl reaction volume containing 111nM GST-AKT-3 (diluted in 25 mM Hepes, pH 7.0, containing 15 mM $MgCl_2$, 1 mM DTT), 0.75 FM Biotinylated Histone H2B, 2nM [γ-^{33}P]-ATP and any agents under test. The reaction was terminated by addition of 100 Fl Stop mix (50 FM ATP, 5 mM EDTA, 0.1% BSA, 0.1% Triton X-100 and 7.5 mg/ml Streptavidin coated PVT SPA beads). After allowing the beads to settle for 30 minutes, the assay mixture was counted in a microtiterplate scintillation counter. The results are illustrated in Figure ~~7.~~[108]6.[109]

AKT3 filter assays were performed at 25~~EC~~[110]°C[111] for 3 hrs in the presence of 25mM Hepes, pH7.0, containing 15 mM $MgCl_2$, 1 mM DTT. Each assay was performed in a 100 Fl reaction volume containing 111 nM GST-AKT-3 (diluted in 25mM Hepes, pH7.0, containing 15 mM $MgCl_2$, 1 mM DTT), 2.5 FM Histone H2B, 2nM [γ-^{33}P]-ATP and any agents under test. The reaction was terminated by addition of 100 Fl 75 mM H_3PO_4. 90Fl of the assay mixture was filtered through Phosphocellulose cation exchange paper. After five times washing with 75 FM H_3PO_4, the filterpaper was counted in a microtiterplate scintillation counter. The results are illustrated in Figure 8.

RESULTS

Molecular cloning of human Akt-3.

Similarity searching of the LifeSeq™ and EMBL databases using the rat Akt-3 sequence as a query sequence yielded several human EST sequences which encoded part of the human homologue of rat Akt-3. Using the DNA sequence information in the databases, we were able in subsequent 3' RACE experiments to deduce the complete cDNA sequence for the human Akt-3 (~~Figure 1).~~[112]SEQ ID NO:1, coding DNA is provided as

SEQ ID NO:2).[113] The obtained cDNA sequence encoded a protein of 479 amino acid residues (SEQ ID NO:3) [114]with a calculated molecular mass of 55770 Da. The first 451 amino acids of the human Akt-3 protein contain only two differences to the corresponding rat sequence (Konishi et al., 1995) - Asp (rat) to Gly (human) at position 10 and Pro (rat) to Ala (human) at position 396 and encode a pleckstrin homology domain, a kinase domain and a COOH-terminal ~~Atail=.~~ [115]"tail". The two amino acid residues that are presumed to be phosphorylated upon activation of Akt-3 (Thr^{305} and Ser^{472}) are in bold and marked with an asterisk. The COOH-terminal part of the human Akt-3 protein that differs with the rat homologue extends from amino acid 452 through amino acid 479.[116]

The predicted Akt-3 (Figure ~~2~~[117]1[118]) protein shows significant similarity with Akt-1 (Jones et al, 1991; 83.6% identity; 87.8% similarity) and with Akt-2 (Cheng et al., 1992; 78% identity; 84.3% similarity).The COOH-terminal `tail= has been observed in both human and rat Akt-1 and Akt-2 proteins, but it is apparently truncated in the only other reported Akt-3 sequence (rat Akt-3, Konishi et al., 1995; accession number D49836). 3'RACE experiments performed on human cDNAs derived from different tissues did not yield evidence for the existence of a shorter form of Akt-3 that would be analogous to the rat Akt-3 (data not shown). The tail in human Akt-3 comprises 28 amino acid residues (YDEDGMDCMDNERRPHFPQF**S**YSASGRE) that replace 3 amino acid residues in the rat sequence (CPL). The tail in human Akt-3 contains a serine residue at position 472 (shown in bold) that corresponds to Ser^{473} in Akt-1 or Ser^{474} in Akt-2. Phosphorylation of Ser^{473} and Ser^{474} has previously been implicated in the activation of Akt-1 and Akt-2, respectively (Alessi et al., 1996; Meier et al.,1997). Thr^{308} (in the kinase domain) has

also been implicated in the activation of Akt-1 and this residue is also conserved in human Akt-3 (Thr^{305}).

Characterisation of Akt-3 activity.

To characterise the enzymatic activity of Akt-3, we expressed and purified the recombinant enzyme as a GST fusion protein. Analysis of the purified product by SDS-PAGE indicated the protein was apparently > 90% pure. The purified enzyme was able to phosphorylate histone H2B (~~figure 3~~[119]Figure 2[120]), and no phosphorylation was observed using recombinant GST alone. Previously, the enzymatic activity of Akt-1 has been shown to be increased by phosphorylation of Thr^{308} and Ser^{473}, and mutation of both these residues to Asp (to mimic phosphorylation) synergistically activates Akt-1 (Alessi et al., 1996). To investigate whether Akt-3 is similarly regulated, GST-fusion proteins in which either Thr^{305} or Ser^{472} (corresponding to Thr^{308} and Ser^{473} in Akt-1) or both Thr^{305} and Ser^{472} had been mutated to Asp were expressed and assayed in comparison to the wild-type enzyme. Mutation of Thr^{305} to Asp (~~AT~~[121]"T[122]305D≅[123]„[124]) resulted in a 2.0-fold increase in the initial rate of phosphorylation of histone H2B, whereas mutation of Ser^{472} to Asp(S472D≅[125]„[126]) increased the initial rate only 1.4 fold (Figure ~~3~~[127]2[128]A). When both Thr^{305} and Ser^{472} (~~AT~~[129]"T[130]305D,S472D) were mutated to Asp, a 3.2-fold increase in the initial phosphorylation rate was observed.

To confirm that extracellular stimuli can activate Akt-3 in mammalian cells, Cos-7 cells were transfected with a cDNA encoding Akt-3 fused to a HA tag. Akt-3 activity in HA immunoprecipitates was increased 1.5 and 1.9 fold (n=2) following stimulation with IGF-1 (10 ng/ml).

To further confirm that extracellular stimuli can activate Akt-3 in mammalian cells, HEK-293 cells were transfected with a cDNA encoding Akt-3 fused to a HA epitope tag. Upon treatment with IGF, Akt-3 activity in anti-HA immunoprecipitates (Figure ~~3~~[131]2[132]B) was increased almost 60-fold above that in untransfected cells (Figure ~~3~~[133]2[134]C). Akt variants in which Thr^{305} and Ser^{472} were mutated to alanine were refractory to activation by IGF. Consistent with this, Western blotting with a Ser^{472} phosphospecific antibody of HA immunoprecipitates from cells stimulated with IGF demonstrated that Ser^{472} was phosphorylated following stimulation with IGF (Figure ~~3~~[135]2[136]B). In addition, activation of Akt-3 was inhibited by prior treatment with CY29 4002 (100 FM, 94% inhibition), data not shown).

To characterise human Akt-3 further, we investigated the ability of a range of Ser/Thr kinase inhibitors to inhibit Akt-3. These included Go 6976, GF-109203X (both protein kinase C (PKC) inhibitors); H-85, H-88, H-89 and KT5720 (protein kinase A (PKA) inhibitors), KN-62 (Ca^{+2}/Calmodulin dependent kinase inhibitor) and PD 98059 (MEK inhibitor). When tested at a concentration of 10 FM these compounds had no significant effect on the activity of the T305D,S472D variant of Akt-3. However, the broad spectrum kinase inhibitor staurosporine (IC$_{50}$= 2.0 $\pm$ 0.3 FM) and the PKC inhibitor Ro 31-8220 (IC$_{50}$=3.2 $\pm$ 1.0 FM) inhibited the T305D,S472D variant of Akt-3 (Figure 4).

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Chromosomal localisation of Akt-3.

The complete coding sequence of Akt-3 was used as a probe for FISH analysis. Under the conditions used, the hybridisation efficiency was approximately 75% for this probe (among 100 checked mitotic figures, 75 of them showed signals on one pair of the chromosomes). Since the DAPI-banding was used to identify the specific chromosome, the assignment between the signal from the probe and the long arm of chromosome 1 was obtained. The detailed position was further determined in the diagram based upon the summary from 10 photographs (Figure ~~5A~~[138]4[139]). There was no additional locus picked by FISH detection under the conditions used, therefore, it was concluded that Akt-3 is located at human chromosome 1, region q43-q44. ~~An example of the mapping results is presented in Figure 5B.~~[140] FISH signals were localized to chromosome 1 on the samples tested. The mitotic figures stained with 4',6- diamidino-2-phenylindole to identify chromosome 1.[141]

Tissue distribution of Akt-3 mRNA.

Northern blot analysis was performed on mRNA derived from different human tissues. Akt-3 mRNA was detected as two transcripts of approximately 4.5 kb and 7.5 kb, showing similar patterns of expression (Fig. 6A). Akt-3 mRNA was expressed in a range of tissues, most prominently in brain. Similarly, rat Akt-3 was detected as multiple transcripts most highly expressed in brain (Konishi et al., 1995). The weakest expression of Akt-3 was observed in two insulin-responsive tissues, skeletal muscle and liver. Akt-3 was also expressed in a number of cancer cell lines including SW480 colorectal adenocarcinoma, A549 lung carcinoma and G361 Melanoma (data not shown).

To confirm the Northern blot analysis, PCR reactions were performed with Akt-3 specific and G3PDH-specific

(internal control) primers on cDNAs derived from different human tissues (Fig. 6B). The Akt-3 message was present in every tissue tested, since a specific 425 bp fragment was amplified in every cDNA after 30 cycles of PCR. Akt-3 mRNA expression was high in placenta, ovary and spleen. Moderate expression was seen in brain, heart, kidney, colon, prostate, small intestine and testis. Lowest expression was in liver, lung, pancreas, skeletal muscle, peripheral blood leukocytes and thymus. In tumor cell lines (Figure ~~6~~[142]5[143]C), Akt-3 mRNA expression was relatively high in HT-1080 bone fibrosarcoma cells, in SaOS-2 osteosarcoma and in JURKAT T-cell leukemia cells (Akt-3 band detectable after 30 cycles of PCR). Caco-2 colorectal adenocarcinoma, T84 colorectal carcinoma, MCF-7 breast adenocarcinoma and SK-N-MC neuroblastoma cells show Akt-3 mRNA expression after 35 cycles of PCR. In T-47D breast ductal gland carcinoma and HepG2 hepatoblastoma, expression of Akt-3 mRNA is very low or absent (no signal detectable after 35 cycles of PCR).

Akt-1 and Akt-2 have been identified in several species. Human (Jones et al., 1991; Coffer et al 1991), mouse (Bellacosa et al., 1993) and bovine (Coffer & Woodgett, 1991) Akt-1 clones have been reported, whereas human (Cheng et al., 1992) mouse (Altomare et al., 1995) and rat (Konishi et al., 1994) clones of Akt-2 have been identified. However, Akt-3 has only been previously identified in rat (Konishi et al, 1995). The present inventors have identified the human isoform of Akt-3. Although human Akt-3 shows considerable similarity to human Akt-1 and Akt-2, the discovery of human Akt-3 is particularly significant because the cDNA sequence encodes a COOH-terminal Atail≅ which includes a phosphorylation site implicated in the activation of Akt-1 and Akt-2 (Alessi et al., 1996; Meier et al., 1997). This tail is absent from the predicted rat amino acid sequence. Human Akt-3 appears to be activated by phosphorylation

in a similar fashion as Akt-1 and Akt-2. However, its expression profile suggests that the principal function of this enzyme is not in regulating responses to insulin.

The sequence which has been identified represents the human homologue of Akt-3. This assignment is based on the >99% identity between the rat and human Akt-3 protein sequences. With the exception of the COOH-terminal tail seen in human Akt-3, there are only 2 amino acid differences (Gly^{10} and Ala^{396} in human Akt-3) between the rat and human Akt-3 proteins. Alignment of all the previously described Akt sequences demonstrates that Gly^{10} and Ala^{396} in the human protein correspond to Gly and Ala residues respectively in the Akt-1 and Akt-2 sequences identified from other species. Further evidence that we have identified the Akt-3 isoform comes from the presence of isotype-specific sequences represented by human Akt-3 residues 47-49 (LPY), 118-122 (NCSPT) and 139-141 (HHK). For each isotype, these sequences are conserved between species, but differ between the isotypes.

The human Akt-3 cDNA sequence was predicted to encode a NH_2-terminal pleckstrin homology (PH) domain (Musacchio et al., 1993) and a COOH-terminal kinase domain. A striking difference between the human and rat Akt-3 protein sequence (Konishi, et al.,1995) is the presence of a COOH-terminal Atail≅ comprising 74 residues after the kinase domain. The last 28 amino acid residues in human Akt-3 are absent from the rat Akt-3 sequence. We were unable to identify human cDNA sequences that encoded a similar truncation, despite conducting RACE experiments using cDNA from several different human tissues. The region in human Akt-3 that is absent from rat Akt-3 encompasses a stretch of 10 residues (residues 467-476 in human Akt-3) which are identical to the corresponding region of human Akt-1 and Akt-2. This suggests that the tail observed

in human Akt-3 is authentic. The significance of the difference observed in the rat Akt-3 tail region remains to be investigated. However, the human Akt-3 COOH-terminal sequence includes Ser^{472}, which corresponds to Ser^{473} in Akt-1. Phosphorylation of Ser^{473} has been shown to lead to a 5-fold increase in the activity of Akt-1, whereas a 20-25 fold increase of Akt-1 activity is observed if both Ser^{473} and Thr^{308} are phosphorylated (Alessi et al., 1996). Thus, our observation that Ser^{472} is present in human Akt-3 is significant, because it suggests that human Akt-3 is potentially regulated in a manner similar to Akt-1 and Akt-2. Whether rat Akt-3 is regulated in a different fashion remains to be resolved.

The kinase and PH domains in Akt-3 show homology to the consensus PH and kinase domain sequences (Musacchio et al., 1993; Hanks & Hunter 1995). The PH domain of human Akt-3 is 77% and 86% identical to the PH domains in Akt-1 and Akt-2, respectively, while the kinase domain of Akt-3 is 88% and 87% identical to the kinase domain of Akt-1 and Akt-2, respectively. The high conservation of the PH domain may indicate an Akt-specific function, because PH domains are often highly divergent (Musacchio et al, 1993). Apart from binding phosphoinositides, the PH domain of Akt has been shown to mediate interactions between Akt and PKC (Konishi, et al.,1995) as well as directing the formation of multimeric Akt complexes (Datta et al, 1995). In contrast, the region between the PH domain and the kinase domain is poorly conserved between the human Akt-1, Akt-2 and Akt-3 sequences, and this region is also important for mediating the formation of multimeric Akt complexes (Datta et al, 1995). This raises an interesting issue - whether the sequence NH_2-terminal to the kinase domain of Akt-3 mediates the interaction with binding partners that are unique to Akt-3 or that bind to multiple Akt isoforms.

To verify that the predicted kinase domain was catalytically active, we expressed Akt-3 as a GST fusion protein in *E. coli*. The purified protein was able to phosphorylate an exogenous substrate, whereas no catalytic activity was observed using GST in place of GST-Akt-3. To confirm that Akt-3 is indeed regulated in a manner akin to Akt-1 and Akt-2, we mutated Thr^{305} and Ser^{473}, either separately or jointly, to Asp. This strategy has previously been shown to faithfully mimic the effect of phosphorylation of these residues in Akt-1 (Alessi et al., 1996). Mutation of either of these residues resulted in increased activity, although the increase was less than that observed with Akt-1 (Alessi et al., 1996). Additionally, we did not observe a synergistic activation of Akt-3 by mutation of both Thr^{305} and Ser^{473}. In contrast, when both the corresponding residues were simultaneously mutated to Asp in Akt-1, synergistic activation was observed (Alessi et al, 1996). The apparent quantitative differences between Akt-1 and Akt-3 may reflect true differences in the regulation of these two isoforms, or it may be due to other factors such as the different expression system used. In the present study Akt-3 was expressed as a GST fusion protein in *E. coli*, whereas Akt-1 activity was studied using an HA-tagged protein expressed in COS cells. Nevertheless, our results demonstrate that Akt-3 is qualitatively regulated in a fashion similar to Akt-1. Previous work has also shown that activation of Akt is dependent upon PI 3-kinase to generate 3-phosphoinositides that bind the PH domain of Akt, promote translocation of Akt to the plasma membrane and facilitate the phosphorylation of Akt by upstream kinases (reviewed in Alessi & Cohen, 1998; Coffer et al., 1998). Our observation that the T305D/S472D mutant of Akt-3 is more active than the wild type enzyme (Figure ~~3~~[144]2[145]), when measured in the absence of 3-phosphoinositides, suggests that after phosphorylation Akt-3 becomes (at least partially)

independent of phosphoinositide binding.

The structure of the catalytic domain of Akt is closely related to protein kinase A and protein kinase C. Indeed, a BLAST search of the SwissProt data base revealed that the most closely related kinases (other than the different Akt isoforms) include several protein kinase C isozymes. This prompted us to investigate whether existing inhibitors of PKA or PKC, as well as other serine/threonine kinase inhibitors, could be used as inhibitors of Akt-3. Of the compounds tested, only staurosporine and the structurally related compound Ro 31-8220 both potently inhibited Akt-3. Staurosporine is a non-selective kinase inhibitor, whereas Ro 31-8220 is a more selective PKC inhibitor (Davis, et al.,1992). Although Ro 31-8220 is an approximately 100-fold more potent (IC_{50}.10 nM; Davis, et al.,1992) inhibitor of PKC than of Akt-3, this observation cautions that experiments using high concentrations of Ro 31-8820 may affect Akt-3. In contrast to staurosporine and Ro 31-8220, two other PKC inhibitors and three other PKA inhibitors did not inhibit Akt-3. This suggests that although Akt-3 is closely related in sequence to PKC, it may be possible to find selective inhibitors of Akt.

The observation that Akt-3 is activated by IGF-1 suggests that Akt-3 may play a role in regulating cell survival. Akt-3 potentially may suppress apoptosis in tumor cells. One concern in using Akt as a target for drug development in cancer is that Akt plays a role in insulin signalling (reviewed in Sheperd et al, 1998). Thus, inhibitors of Akt may induce symptoms observed in patients with diabetes. One solution that has been proposed is to develop selective inhibitors of Akt-2 (Walker et al, 1998). This is based in part on the observation that Akt-1 is strongly activated by insulin in rat hepatocytes and skeletal muscle, whereas Akt-2 is only weakly activated by insulin in

these tissues. However, rat Akt-3 appears to be even more weakly activated by insulin in these tissues (Walker et al, 1998), and in this study we have shown that Akt-3 mRNA is expressed only at low levels in human liver and skeletal muscle, which are insulin responsive tissues. This suggests that selective inhibitors of Akt-3 could have even less potential to cause symptoms similar to those seen in patients with diabetes than do inhibitors of Akt-2. The localisation of human Akt-3 to human chromosome 1q43-44 is also interesting, as patients with haematological cancers have been reported with chromosomal abnormalities in this region (Mitelman et al, 1997). Although the significance of the latter observation is debatable, as chromosomal abnormalities at numerous loci have been observed in patients with haematological cancers, the results presented here indicate that Akt-3 may prove to be an important target for the development of novel therapeutics for the treatment of cancer.

SEQUENCE LISTING

1. Sequence ID No. 1 corresponds to the nucleotide sequence of Akt-~~3 illustrated in Figure 1.~~[146]3.[147]

2. Sequence ID No. 2 corresponds to from nucleotide position 11 to 1447 of the nucleic acid sequence of Akt-~~3 illustrated in Figure 1.~~[148]3.[149]

3. Sequence ID No. 3 corresponds to the amino acid sequence of Akt-3 illustrated in ~~Figures 1 and 2.~~[150]Figure 1.[151]

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CLAIMS

1. A nucleic acid molecule encoding human Akt-3 protein or a functional equivalent, derivative or bioprecursor thereof, comprising the amino acid sequence illustrated in SEQ ID No. 3.

2. A nucleic acid molecule according to claim 1 which is a DNA molecule, and preferably cDNA.

3. A nucleic acid molecule according to claim 1 or 2 comprising the nucleotide sequence illustrated in SEQ ID No. 1.

4. A nucleic acid molecule according to claim 1 or 2 comprising the nucleotide sequence in SEQ ID No. 2.

5. An antisense molecule capable of hybridising to the molecule according to any of claims 1 to 4 under high stringency conditions.

6. A human Akt-3 protein or a functional equivalent, derivative or bioprecursor thereof, comprising an amino acid sequence as illustrated in SEQ ID No. 3.

7. A human Akt-3 protein or a functional equivalent, derivative or bioprecursor thereof encoded by a nucleic acid molecule according to any of claims 1 to 4.

8. A human Akt-3 protein according to claim 7 comprising the amino acid sequence as illustrated in SEQ ID No. 3.

9. An expression vector comprising a nucleic acid molecule according to claim 2 or 3.

10. An expression vector according to claim 9 comprising an inducible promoter.

11. An expression vector according to claim 9 or 10 comprising a sequence encoding a reporter molecule.

12. A nucleic acid molecule according to any of claims 1 to 5 for use as a medicament.

13. Use of a nucleic acid molecule according to any of claims 1 to 5 in the preparation of a medicament for treating cancer.

14. A human Akt-3 protein according to any of claims 6 to 8 for use as a medicament.

15. Use of a human Akt-3 protein according to any of claims 6 to 8 in the preparation of a medicament for treating cancer.

16. A pharmaceutical composition comprising a nucleic acid molecule according to any of claims 1 to 5 or a human Akt-3 protein according to any of claims 6 to 8 together with a pharmaceutically acceptable carrier diluent or excipient therefor.

17. A host cell or organism, transformed or transfected with an expression vector according to any of claims 9 to 11.

18. A transgenic cell, tissue or organism comprising a transgene capable of expressing a human Akt-3 protein according to any of claims 6 to 8.

19. A human Akt-3 protein expressed from the cell or organism according to claim 17 or 18.

20. An antibody capable of binding to a human Akt-3 protein or an epitope thereof according to any of claims 6 to 8.

21. An antibody according to claim 20 which is a monoclonal antibody.

22. An antibody according to claim 20 or 21 for use as a medicament.

23. Use of an antibody according to claim 20 or 21 in the preparation of a medicament for treating cancer, or other diseases or conditions associated with human Akt-3 protein expression.

24. A kit for detecting human Akt-3 protein in a sample which protein comprises a sequence according to any of claims 6 to 8, said kit comprising an antibody according to claim 20 or 21 and means for contacting said antibody with said sample.

25. A method of identifying compounds which selectively inhibit human Akt-3 protein mediated promotion of cell survival said method comprising:

i) providing a cell transformed with an expression vector activating the Akt-3 pathway that survives in the presence or absence of a survival factor compared to a control cell which has not been transformed with said vector and will die in the absence of said survival factor,

ii) contacting each of said cells with a test compound following the removal of said cells from said survival factor, wherein death of said transformed cell is indicative of selective inhibition of said compound on the survival promoting human Akt-3 pathway.

26. A method of identifying compounds which selectively inhibit human Akt-3 protein mediated promotion of cell survival, said method comprising:

i) providing a cell transformed with an expression vector activating the Akt-3 pathway in addition to a control cell which

has not been transformed with said vector,

ii) contacting each of said cells with a death inducing agent, whereby death of said control cell and survival of said transformed cell is indicative of the survival promoting activity of the activated Akt-3 pathway,

iii) contacting said transformed cell with a test compound, wherein death of said cell is indicative of selective inhibition of said compound on the survival promoting human Akt-3 pathway.

27. A compound identifiable according to the method of claims 25 or 26.

28. A compound according to claim 27 for use as a medicament.

29. Use of a compound according to claim 27 in the manufacture of a medicament for treating diseases associated with human Akt-3 protein expression.

30. A method of identifying agents which influence the activity of a human Akt-3 protein according to any of claims 6 to 8, said method comprising contacting said human Akt-3 protein with a substrate therefor in the presence of a test compound and a phosphate source, and monitoring for any phosphorylation of said substrate.

31. A method according to claim 30 wherein said Akt-3 protein is provided as a fusion or epitope tagged protein having a domain capable of phosphorylating a known substrate.

32. A method according to claim 30 wherein said Akt-3 protein is provided as a fusion molecule of GST and human Akt-3.

33. A method of identifying agents which influence the activity of a human Akt-3 protein according to any of claims 6 to 8, said method comprising contacting a phospholipid or a surrogate or functional equivalent thereof, with a PH domain of a human Akt-3 protein according to any of claims 6 to 8 in the presence of an agent to be tested and monitoring for any binding of said phospholipid, surrogate or functional equivalent thereof with said PH domain of said Akt-3 protein.

34. A method according to claim 33 wherein said phospholipid comprises phosphatidylinositol 3,4,5-triphosphate.

35. An agent identifiable according to the method of claim 33 or 34.

36. An agent according to claim 35 for use as a medicament.

37. Use of an agent according to claim 35 in the preparation of a medicament for treating diseases associated with human Akt-3 expression.

38. A method of treating diseases associated with human Akt-3 activity said method comprising administering to an individual suffering from said disease a compound that inhibits the function and/or expression of a human Akt-3 protein according to any of claims 6 to 8, in a sufficient concentration to reduce the symptoms of said disease.

39. A method according to claim 38 wherein said compound is any of an antisense molecule according to claim 5, an antibody according to claim 21 or 22, a compound according to claim 27 or an agent according to claim 35.

40. A method for making a pharmaceutical formulation for the treatment of diseases associated with human Akt-3 protein expression, said method comprising:

a) contacting candidate compounds with a host cell that expresses human Akt-3 protein,

b) selecting a compound identified in step a) which binds to human Akt-3 protein,

c) manufacturing bulk quantities of the compound selected in step b), and

d) formulating the compound manufactured in step c) in a pharmaceutically acceptable carrier.

41. A method of identifying a compound which modulates Akt-3 kinase activity, comprising:

a) contacting said Akt-3 with a substrate thereof in the presence of a radiolabelled phosphate source, and the compound to be tested,

b) stopping the reaction by the addition of kinase inhibitor in the presence of SPA beads,

c) monitoring the signal from said beads compared to a control which has not been contacted with said compound.

42. A method of identifying a compound which modulates Akt-3 activity, comprising:

a) contacting said Akt-3 with a substrate thereof in the presence of a radiolabelled phosphate source, and the compound to be tested,

b) stopping the reaction,

c) filtering the reaction mixture through phosphocellulose cation exchange paper, and

d) monitoring the signal from said filter paper compared to a control which has not been contacted with said compound.

ABSTRACT

HUMAN AKT-3

There is disclosed a nucleic acid molecule encoding human Akt-3 protein or a functional equivalent or bioprecursor thereof comprising the amino acid sequence illustrated in Sequence ID No. 3. The human Akt-3 protein itself also forms part of the invention. The nucleic acid molecule and the human Akt-3 protein may themselves be used as medicaments, or in the preparation of medicaments for treating cancer, in their own right or in the form of a pharmaceutically acceptable carrier, diluent or excipient thereof. Further disclosed are methods of identifying agents which influence the activity of a human Akt-3 protein.

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